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Mitotic index and aneuploidy variation in growing day 2- to day 7- IVP bovine embryos of the Agerolese breed of cattle

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The present study reports on the variation of the mitotic index in growing day 2- to day 7- IVP bovine embryos of the Agerolese breed of cattle. After IVM (24 h), COCs were transferred into 25/well with 300 µl IVF-TALP and covered with mineral oil. Frozen sperm from a bull were selected by centrifugation on a Percoll discontinuous gradient (45–80 %). The pellet was diluted in IVF medium and added to the COCs at the concentration of  $1 \times 10^6$  sperm/mL. Gametes were co-incubated for 20–22 h at 39 °C, in 5 % CO<sub>2</sub> in air. After co-incubation, presumptive zygotes were vortexed to remove cumulus cells and randomly allocated in six groups, each into 400 µl of SOF medium, with 30 µl/ml essential amino acids, 10 µl/ml non-essential amino acids, 0.34 mM tri-sodium citrate, 2.77 mM myo-inositol, and 5 % BS. Zygotes were incubated in a humidified mixture of 5 % CO<sub>2</sub>, 7 % O<sub>2</sub> and 88% N<sub>2</sub> in air at 39 °C for 20–22 h. Starting on Day 0 ( IVF day), embryos were taken out of the incubator at day 2(48 h), 3 (72 h), 4 (96 h), 5 (120 h), 6(144 h) and 7(168 h), examined under a stereomicroscope, treated in a lysing buffer (0.01 N HCl, 0.1 % Tween 20) for 30 s, transferred in a small droplet to a precleaned slide and fixed with methanolacetic acid (3:1). Out of 178 embryos (3,100 cells) analyzed, the mitotic index was 19.6 % (28/143 cells) at day 2, 18.6 % (41/221 cells) at day 3, 10.3 % (27/263 cells) at day 4, 7.1 % (26/367 cells) at day 5, 0.7 % (5/681 cells) at day 6 and 0.6 (9/1425 cells) at day 7. FISH analysis is undergoing and will be reported elsewhere.

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