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Neutral Red as viability marker for isolated, bovine pre-antral ovarian follicles: optimal concentration and incubation time.

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Fertility preservation strategies warrant the need for non-invasive techniques to assess pre-antral follicle (PA) viability. Neutral Red (NR) is considered a non-toxic dye, incorporated by viable follicles (Chambers et al., 2010). We determined the lowest concentration and shortest exposure time needed for optimal PA viability screening for isolated bovine follicles retrieved after slaughter. Isolated PA (900 in total, divided over 6 replicates) were cultured in DMEM/Ham's F12 (Culture Medium - CM) for 4 days (38.5°C, 5% CO₂). On d0, d2 and d4, follicles were stained for 120 minutes by adding Neutral Red medium (NRm = CM with different concentrations NR) to obtain final NR concentrations of 0, 0.0025, 0.025, 0.25, 2.5 and 25µg/mL NR. PA viability assessment is performed by counting numbers of stained/non-stained PA every 30 minutes. Because follicles only stained positive in the 2.5 to 25µg/mL NR concentration range, the trial was repeated with 0, 2.5, 5, 10, 15, 20 and 25µg/mL NR respectively. Following a binary logistic regression analysis with staining result (yes/no) versus log-concentration, a probability model could be fitted, indicating that the proportion of stained follicles remained stable when 15µg/mL NR was used for 30 minutes, without compromising follicular health and short-term developmental competence of PA after 4 days of culture.