Assessing Cryotolerance in Rabbit Embryos Across Three Consecutive Generations: The Impact of Vitrification on Offspring

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Cryopreservation is a method for long-term embryo storage, potentially preserving genetic diversity. However, its effectiveness in maintaining viability over extended periods and the cryotolerance of embryos across successive generations are not well understood. This knowledge is crucial for applications such as regenerating live animals and restoring lost breeds. This study focused on assessing the cryotolerance of embryos over three consecutive generations in rabbits, aiming for successful offspring regeneration. Natural conceived females were super-stimulated (3 µg of Corifollitropin alfa) and artificial inseminated with monospermic sperm dose ($20x10^6$ millions) and late morulae and early blastocysts were recovered and vitrified using a French ministraw and a two-step vitrification protocol (EQ: 10% DMSO and 10% EG for 2 minutes; V: 20% DMSO and 20% EG for less than 1 minute). After warming, embryos (14-16 embryos by female) were transferred to natural conceived foster mothers to stablish F1 population. Once the animals from the F1 population reached adulthood, females and males were bred, deliberately avoiding mating between animals with common grandparents to reduce inbreeding. One-two female from each sire family was super-stimulated to produce embryos to establish the F2 generation. This process was repeated to generate the F3 generation. A total of 1,104 vitrified embryos were transferred; 100 for F1, 310 for F2, and 694 for F3 to 7, 18 and 48 naturally conceived females respectively, and the offspring rate was recorded by generation. The offspring rate significantly decreased across generations (65%^{a}, 34%^{b}, and 17%^{c} for F1, F2, and F3, respectively, $p<0.05$). These data suggest that embryos may lose their cryotolerance capability over vitrification generations. The study was funded by the Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital (CIAICO/2022/072).