16. Donor age as a limiting factor in the conservation of endangered cattle breeds through assisted reproductive technologies

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Assisted reproductive technologies (ARTs) and the storage of cryopreserved viable gametes, embryos, and ovarian tissues (cryobanking) hold considerable promise for preserving genetic biodiversity in small and threatened animal populations. Their application extends beyond wildlife conservation, as it is equally critical for safeguarding local livestock populations and preserving their unique genetic traits.

In this context, ARTs and cryobanking were applied to the genetic conservation of Varzese-Ottonese-Tortonese (VOT) cattle, an FAO-listed endangered Italian breed with a total population of 847 individuals. The project involved the preservation of germplasm from 27 female VOT donors: 15 donors underwent on-farm techniques, specifically Multiple Ovulation and Artificial Insemination (MOAI, n=2) or Ovum Pick-Up followed by IVP (OPU, n=13); 12 donors, culled for meat production, age-related issues, and infertility, were subjected to genetic rescue (GR) by IVP. For IVP, VOT bull semen was used according to the breeder association's mating plans. Additionally, from GR, oocytes derived from early antral follicles were retrieved for long-term in vitro culture and subsequent IVM to cryopreserve MII (Barros, Reproduction,165, 221, 2023). Preantral follicles were preserved as ovarian cortex fragments (Alkali, Reproduction, 166, 299, 2023). Extra pieces were histologically processed for morphometric analysis in heifers (20-21 months) and cows (32-153 months). Overall, 142 embryos (MOAI, n=2; OPU, n=43; GR, n=97), 35 MII oocytes, and 120 ovarian cortex fragments were retrieved.

As proof of principle that VOT cryopreserved embryos can sustain cross-bred implantation, 22 grade 1 embryos (OPU, n=19; GR, n=3) were randomly assigned for transfer into synchronized Holstein surrogate dams, successfully resulting in the birth of 4 calves (calving rate: 18%). Notably, full-term pregnancies occurred only with embryos produced by oocyte donors younger than 113 months (4 out of 7 ET, 57%), while no pregnancies resulted from older donors (114-204 months, 0 out of 15 ET). Embryo origin (7/7 vs 12/15 OPU-derived), freezing/thawing procedures, and recipient parity (6/7 vs 12/15 nulliparous for the young and old groups, respectively) were not different, suggesting that advanced maternal age in donor cows negatively impacts embryo quality and implantation efficiency.

The demographic study highlighted critical age distribution, showing that 25.11% of the VOT female population is over 7 years old. Furthermore, morphometric analysis indicated a significant decrease in preantral follicle density with age (heifers: 290.4±144.5; cows: 32.71±6.9 follicles/mm^3, $P<0.005$), complicating the genetic preservation scenario for this breed. Our results indicate the need to prioritize younger females in conservation programs, as advanced age compromises reproductive performance, limits the success of ARTs, and reduces the availability of gametes for cryobanking to exploit the full ovarian reserve.

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