Rescue of Preantral Follicles from Ovaries of Endangered Cattle Breeds Undergoing Genetic Salvage

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The intense selection of a few highly productive breeds over the past 50 years has led to a dramatic decline in the variety of cattle biodiversity. The loss of several local breeds and the limitation in genetic diversity across many bovine species has garnered urgent attention. Italy has a wide variety of local breeds, favored by the richness of territorial environmental diversity. Specifically, the Varzese cattle breed, originating from Lombardy, is characterized by rusticity, resistance to disease, longevity, and high milk quality.
However, since the widespread of cosmopolitan breeds in the 1960s, Varzese cattle declined to the point where they are currently at risk of extinction. Strategic policies and action plans for animal genetic conservation are operative and allowed to partially rescue the breed. Indeed, the headcount of cattle, which stood at 39 in 2001 as per the Cattle Breeder Association Herd book, increased to 827 in 2023, thanks to EU policies and National plans. However, a further consistent increase is not expected due to the lack of interest of most breeders in these cattle. Therefore, intensive ex-situ preservation programs must be pursued to rescue the limited genetic material available through advanced assisted reproductive technologies. Although more challenging and less efficient than bovine sperm cryobanking, technologies that allow the preservation of oocytes will safeguard the only inheritable source of mitochondrial DNA.
In this sense, the bovine ovary is a considerable source of oocytes established during fetal life and organized into follicles at various stages of folliculogenesis. However, preantral follicles, the most abundant quota of the ovarian reserve, remain unexploited. As such, our study aims to provide strategies for rescuing preantral follicles from the ovarian cortex of Varzese cattle.
A pilot study was performed using Holstein-Friesian bovine ovaries as a model to histologically evaluate the follicle density. Ovarian cortical fragments collected from slaughtered heifers (12-24 months old) and cows (40-87 months old) were fixed and submitted to morphometric analyses to quantify the preantral follicle population contained within 1 mm^3 of the ovarian cortex. In parallel, a mechanical isolation protocol was applied to liberate preantral follicles from the same ovary. For this purpose, fragments of the cortex of 0.16 cm^3 were minced, homogenized, and filtered through sieves of decreasing pore sizes to obtain a homogenous population of preantral follicles. Using this procedure the yield of follicles per fragment in the Holstein-Friesian model was 653.25±279.71 and 105.91±89.85 (mean±SD, $p<0.05$), with an efficiency rate per mm^3 of 10.54 and 4.69% in heifers (N=8) and cows (N=11), respectively. Preliminary ovarian reserve quantification was then performed on Varzese heifers (N=2) and cows (N=2) on ovarian fragments, representing the by-products of the dissection of antral follicles used for in vitro embryo production. Our results showed a decrease in ovarian reserve with age. Comparable fragments from the same animals were then cryopreserved to construct a biobank with an estimated number of gametes that may be used in the future for biodiversity restoration, either by grafting, in vitro culture of the ovarian cortex, or follicle isolation and subsequent in vitro folliculogenesis. Beyond the expected decline of the ovarian reserve with age, our approach proposes a paradigm to evaluate the timing for the rescue interventions of female germplasm in biodiversity preservation plans for species at risk of extinction.
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