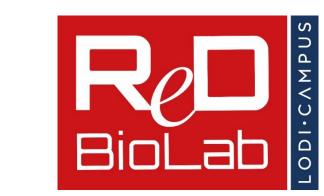
#### **AETE 2025**

**AETE 41<sup>st</sup> Annual Meeting** Cork (IRELAND), September 4<sup>th</sup> – 5<sup>th</sup> 2025





## DONOR AGE AS A LIMITING FACTOR IN THE CONSERVATION OF ENDANGERED CATTLE BREEDS THROUGH ASSISTED REPRODUCTIVE TECHNOLOGIES

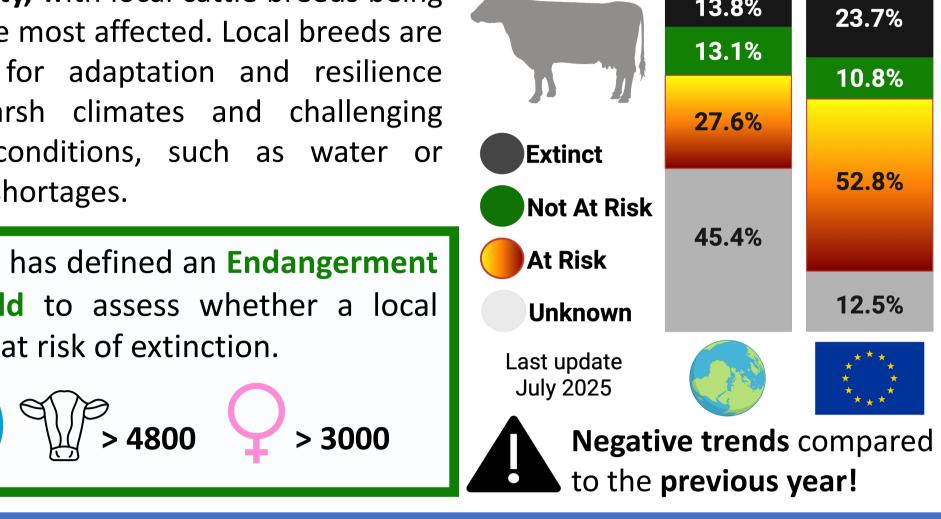
<u>Ludovica Donadini<sup>1</sup></u>, Valentina Lodde<sup>1</sup>, Fernanda Fagali Franchi<sup>1</sup>, Noemi Monferini<sup>1</sup>, Martina Colombo<sup>1</sup>, Isa Mohammed Alkali<sup>1</sup>, Gaia Cecilia Luvoni<sup>1</sup>, Pritha Dey<sup>1</sup>, Roberto Duchi<sup>2</sup>, Paola Turini<sup>2</sup>, Silvia Colleoni<sup>2</sup>, Cesare Galli<sup>2</sup>, Giovanna Lazzari<sup>2</sup>, Federica Franciosi<sup>1</sup>, Alberto Maria Luciano<sup>1</sup>

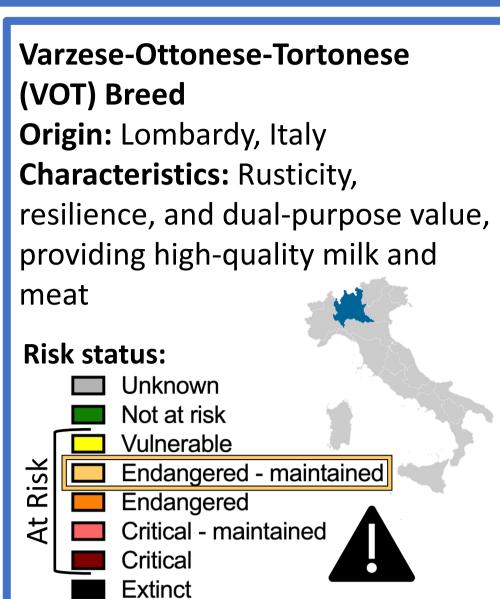
<sup>1</sup>Reproductive and Developmental Biology Laboratory, Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy <sup>2</sup>Avantea srl, Cremona, Italy

### Background

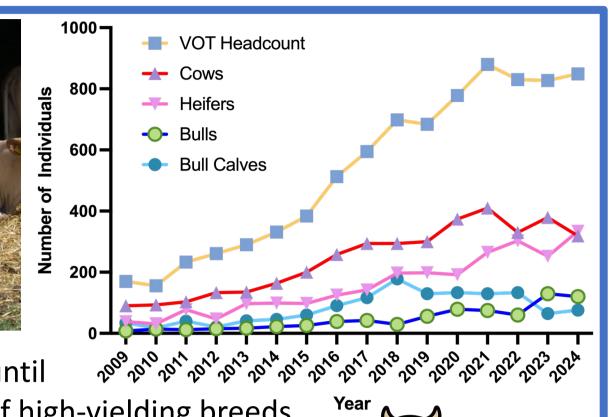
Genetic erosion threatens livestock biodiversity, with local cattle breeds being among the most affected. Local breeds are essential for adaptation and resilience under harsh climates and challenging farming conditions, such as water or resource shortages.

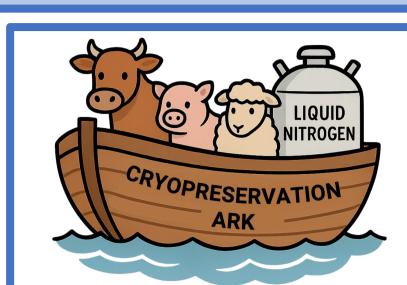
The FAO has defined an Endangerment Threshold to assess whether a local breed is at risk of extinction.











**Assisted** reproductive technologies (ARTs) and cryobanking viable embryos, and tissue ovarian provide significant potential preserving for genetic diversity.

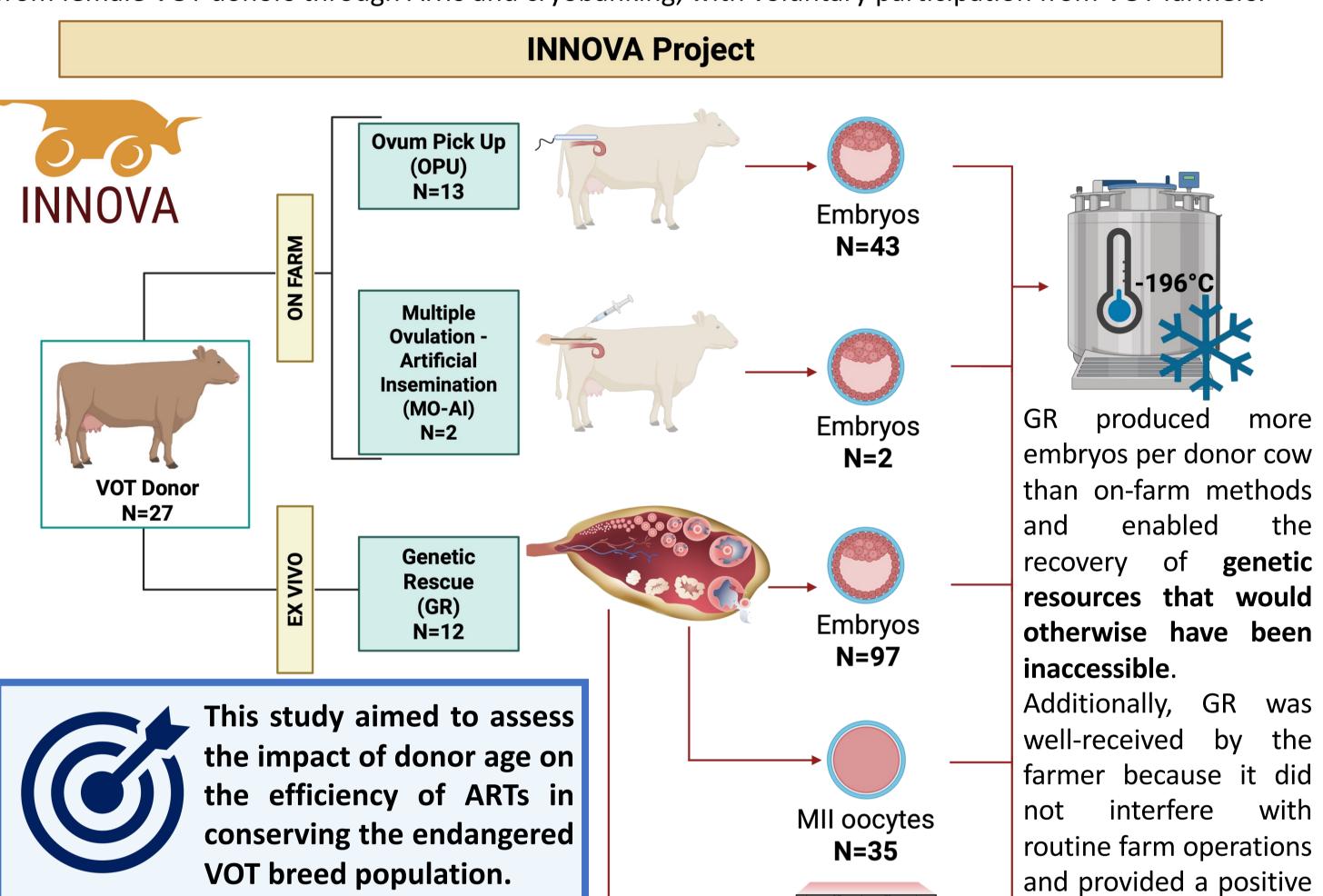
The VOT breed was widespread until the 1960s, but the introduction of high-yielding breeds led to a dramatic decline, reaching a historic low of just **39 animals in 2001**. Thanks to conservation policies, the VOT population has slowly increased, but still far from reaching FAO's threshold.

## **Experimental Design and Aim**

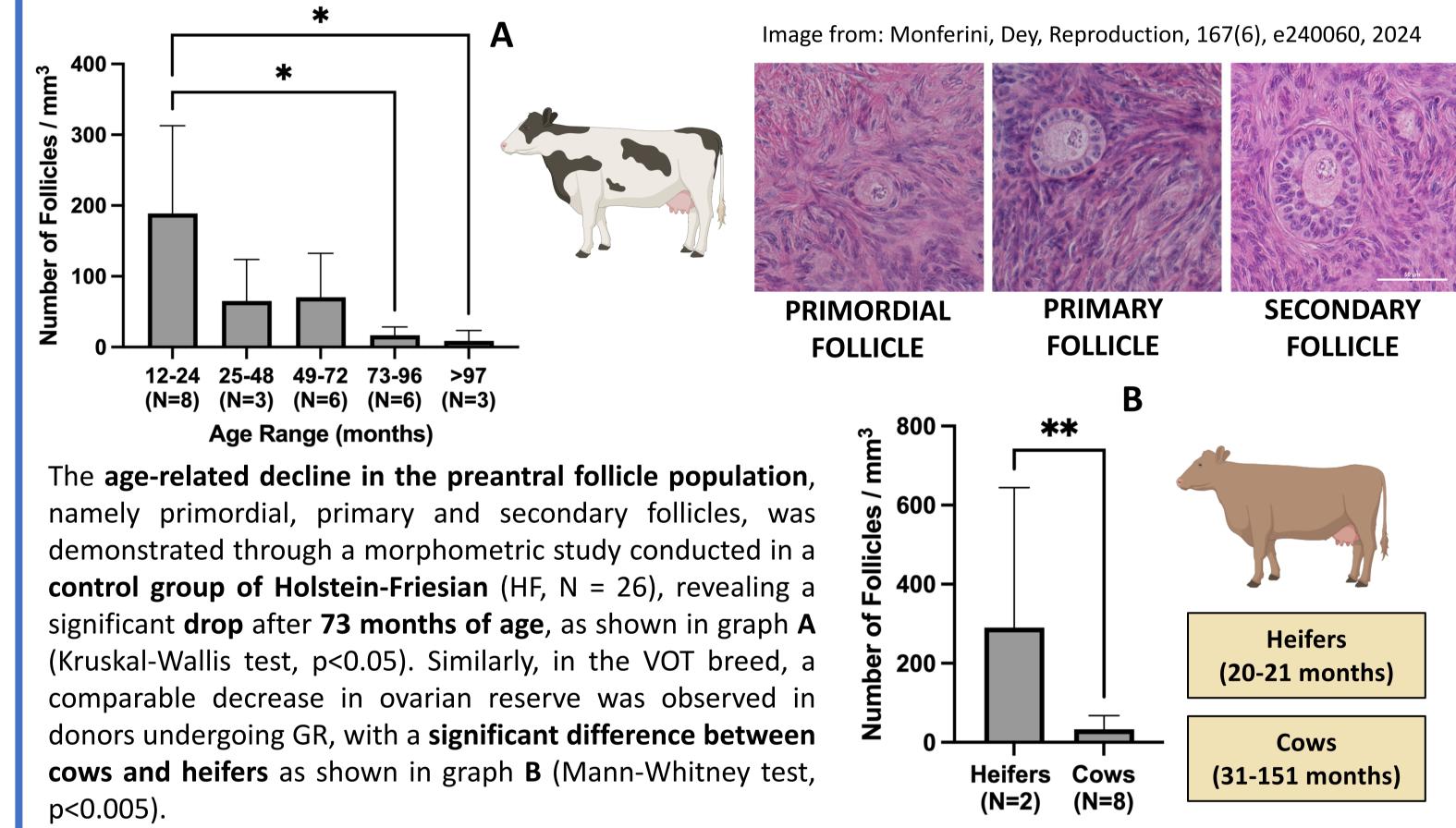
**Local Cattle Breeds Risk Status** 

13.8%

The INNOVA Project is an integrated ex-situ conservation program focused on the retrieval of germplasm from female VOT donors through ARTs and cryobanking, with voluntary participation from VOT farmers.



# **Results 2 - Morphometric Analyses**



## **Results 1 - Embryo Transfer**

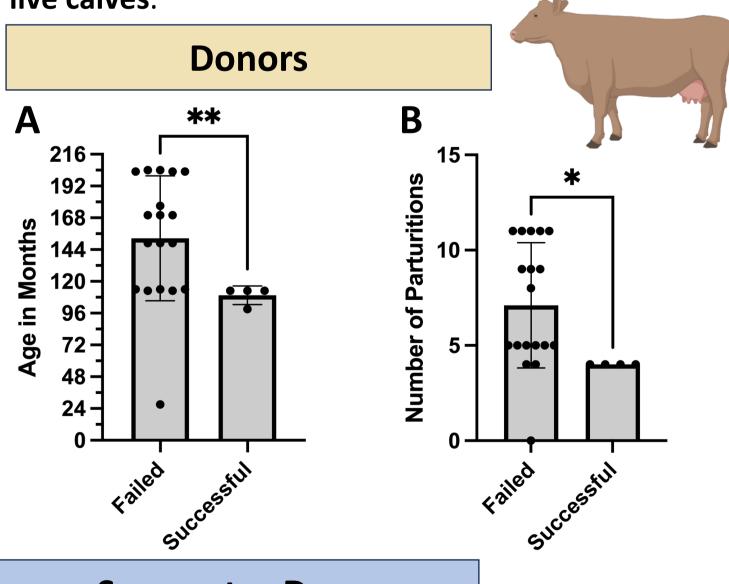
As proof of principle that cryopreserved VOT embryos can support cross-breed implantation, N = 22 IETS grade 1 embryos were randomly transferred into synchronized surrogate dams, successfully resulting in the birth of N = 4 live calves.

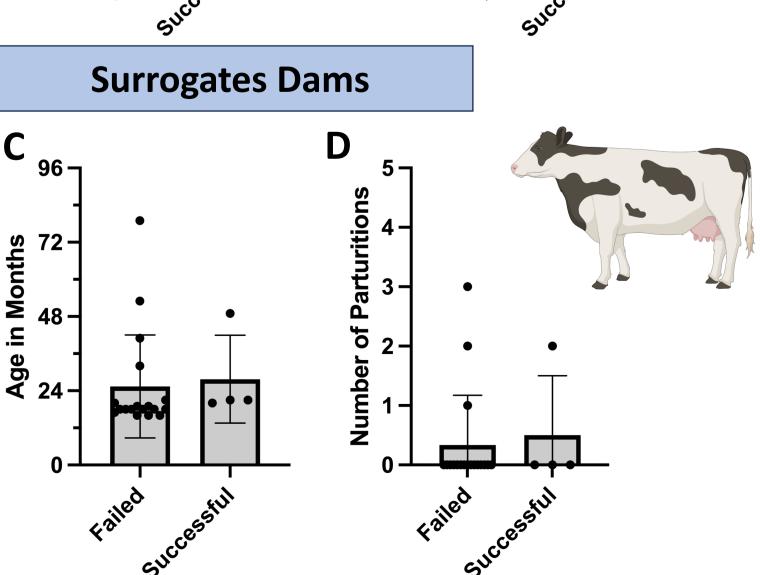
Embryo transfer outcomes, along with

complementary data, were analyzed to

identify age-related constraints on

livestock biodiversity efforts strategies.





# Curious to see the calves? Scan the QR code!

Ovarian cortex

fragments

N = 120

economic benefit.

#### Overall calving rate: 18%

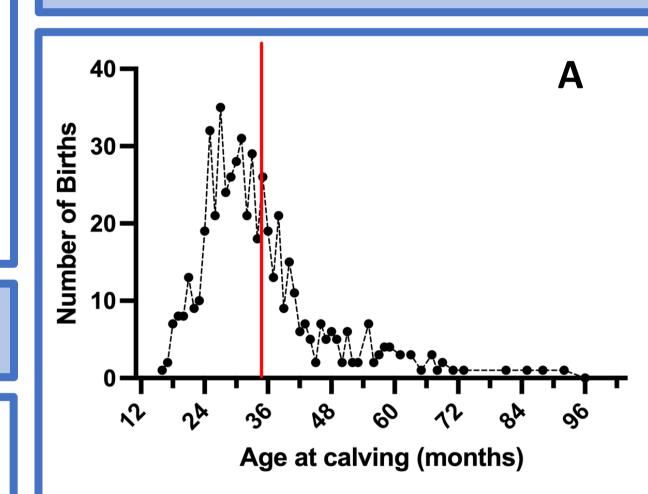
Successful pregnancies occurred only from embryo transfer (ET) derived from donors aged ≤113 months. All pregnancies from older donors failed, as shown in graph A (Mann-Whitney test, p < 0.005). In addition, as shown in graph B, parity also differed significantly between the two groups: the mean parity for successful pregnancies was 4.0, compared to 7.11 ± 3.29 for failed pregnancies (Mann-Whitney test, p<0.05).

In contrast, no significant differences were observed in either age or parity of the between those that surrogate dams successfully carried pregnancies to term and those that did not, as shown in graphs **C** and **D**.



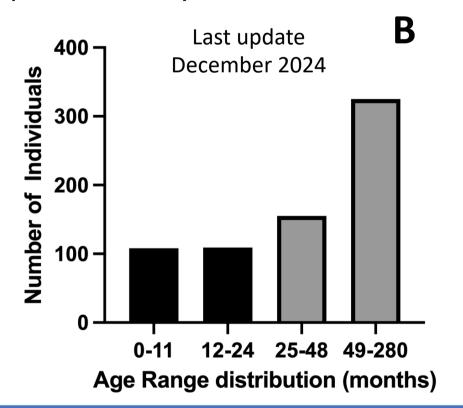
Gestation length, birth weight, and APGAR score were recorded for each calf. The image above shows one of the newborn VOT calves with the surrogate dam and Dr. Duchi, who assisted during delivery.

## **Results 3 - Population Analyses**



The analysis of reproductive records (2010-2025), provided by the Breeders' Association, from N = 550 VOT dams revealed an average age at first calving of 35 months (as shown by the red bar in graph A). Specifically, 86% of these dams calved for the first time after 24 months of age. This average is considerably higher than the commonly recommended in commercial farming systems, likely reflecting management limitations and the extensive nature of the breed's low-input, pasture-based production systems.

Additionally, as shown in graph **B**, the **female population of VOT exhibits** a considerable discrepancy between young (black bars) and adult animals (grey bars). Specifically, 31 % of the population is over 73 months old, when ovarian reserve begins to decline, and 14% is over 113 months, an age linked in this study to a reduced embryo quality. The progressive aging of the population poses a challenge for conservation efforts.



### Conclusions

- Integrated, multi-tool conservation programs that combine different ARTs and cryobanking strategies represent the future of biodiversity preservation, not only for wild species but also for endangered livestock breeds. Specifically, on-farm techniques should always be complemented by ex vivo retrieval methods to maximize the use of genetic resources, as these approaches have proven to be effective.
- ET is a valuable tool to enhance calving opportunities in endangered breeds. However, donor age represents a major limiting factor, significantly affecting calving rate, a trend already observed in a large-scale earlier study that identified 15 years as a critical age for donor cows (Hasler, Theriogenology, 27(1), 139, 1987).
- Follicle density decreases with age in both HF and VOT breeds, with a notable decline observed after six years of age (73 months). Since the VOT female population is considerably aging, early interventions are necessary to improve reproductive efficiency. This involves improving onfarm management to maximize the reproductive potential in heifers.
- Our results indicate that advanced maternal age in donor cows negatively impacts embryo quality and implantation efficiency. Further studies are ongoing to explore how these findings can be directly applied to enhance protocols for genetic rescue and fertility preservation interventions. This knowledge will be vital in refining assisted reproductive strategies aimed at maintaining the reproductive potential of endangered breeds, especially when working with limited and aging genetic resources.









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