



Italian Semen Cryobank for *ex situ in vitro* conservation of poultry autochthonous breeds

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FONDO EUROPEO AGRICOLO PER LO SVILUPPO RURALE: l'Europa investe nelle zone rurali



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MIPAAFT – National Rural Development Measure 10.2 Biodiversity 2017/2023

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TuBAvI Project Conservation of Biodiversity in Italian Poultry Breeds www.pollitaliani.it

Promote and support the conservation of Italian poultry genetic resources providing new data and tools for the implementation of a wide comprehensive national conservation program

- WP1: Phenotipic characterization of local breeds and species
- WP2: Genetic characterization of local breeds and species
- WP3: Genetic and genomic index and breeding management
- WP4: Assessment of inbreeding and genetic diversity
- WP5: Monitoring genetic diversity in local breeds within nucleus populations
- WP6: Assessment of peculiar traits related to disease resistance
- WP7: Cryobanking of semen from Italian chicken and turkey breeds
- WP8: Communication and dissemination



https://ec.europa.eu/agriculture/rural-development-2014-2020_it





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Aim WP7: Semen Cryobank is mainly aimed to support the management of populations *in vivo* conserved and the potential reconstruction of breeds in case of extinction or drastic reduction of their population size.





Activity 2018-2021 Planning and creation of the Semen Cryobank:

- Identification of species-specific semen freezing/thawing reference procedures: chickens and turkeys
- ✓ Drafting Standard Operative Procedures for the implementation of the Cryobank
- ✓ Storage of semen doses from Italian chicken and turkey breeds.

Partners: University of Milan, Silvia Cerolini University of Molise, Nicolaia Iaffaldano









Tutela della biodiversità nelle razze avicole italiane AVICOLA ITALIANA 2nd International workshop within APM NAWA project ScienceNet Cryobanking A life insurance for biodiversity November 6th, 2021



Species-specific freezing/thawing reference procedures **Chicken Semen Turkey Semen** Dilution to 6x109 sperm7mL in Lake diluent (LD) Dilution to 1.5×10^9 sperm/mL in Lake diluent Cooling to 4° C for 25 min added with 0.1M trehalose (LT) Cooling to 4° C for 20 min Further dilution to 3x10⁹ sperm/mL in LP added Further dilution to 1.0×10^9 sperm/mL with (LT) with DMSO 20% e Ficoll 1 mM \checkmark added NMA 2% final concentration Packaging in straws (0.25 mL): 750x10⁶/straw \checkmark Equilibrium at 4° C for 1 min Equilibrium at 4° C for 20 min Packaging in straws (0.25 mL): 250x10⁶/straw Freezing in liquid nitrogen vapors (10 cm height) Freezing in liquid nitrogen vapors (3 cm height) for 10 min \checkmark for 10 min Trnsfer and storage into cryotank \checkmark Trnsfer and storage into cryotank Thaving at 50° C for 10 sec \checkmark Thawing at 5° C for 100 sec Recovery rates after cryopreservation Recovery rates after cryopreservation Viable sperm = 56 %Viable sperm = 52 %Motile sperm = 59 %Motile sperm = 43 %Progressive motile sperm = 50 %Progressive motile sperm = 16 %





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Standard Operative Procedure for the implementation of the Cryobank







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Storage of semen doses from Italian chicken and turkey breeds









| Species | Breed | N.Males | N. straws |
|--------------------|-------------------------|---------|-----------|
| Gallusgallus | Bianca di Saluzzo | 12 | 240 |
| | Bionda Piemontese | 14 | 257 |
| | Mericanel della Brianza | 18 | 62 |
| | Siciliana | 14 | 81 |
| Meleegrisgallopavo | Romagnolo | 5 | 50 |
| | Bronzato | 2 | 13 |
| | Ermellinato di Rovigo | 5 | 11 |







Critical points:

- Low adaptability to semen collection (30-35% donors in chickens)
- Low semen production (pooled samples *vs* ejaculates)
- Chickens more difficult compared to turkeys







Semen quality before and after freezing/thawing in chicken breeds

| Semen parameters | | Breeds | | | | | |
|----------------------|-------------------|-------------------|------------------|------------------|--|--|--|
| (%) | Bianca Saluzzo | Bionda Piem. | Mericanel Bri. | Siciliana | | | |
| Fresh semen | | | | | | | |
| Membrane integrity | 90.91 ± 9.87 | 91.53 ± 10.31 | 96.31 ± 2.24 | 90.23 ± 3.48 | | | |
| Total motility | 90.72 ± 14.65 | 89.35 ± 13.40 | 93.07 ± 7.55 | 84.88 ± 2.85 | | | |
| Progressive motility | 26.63 ± 10.23 | 25.77 ± 9.03 | 30.45 ± 9.41 | 24.62 ± 5.77 | | | |
| Frozen/ thawed semen | | | | | | | |
| Membrane integrity | 26.48 ± 12.57 | 40.16 ± 9.99 | 34.76 ± 7.66 | - | | | |
| Total motility | 26.83 ± 11.57 | 32.49 ± 10.92 | 22.97 ± 1.33 | - | | | |
| Progressive motility | 2.34 ± 2.08 | 3.41 ± 0.97 | 2.70 ± 0.79 | - | | | |



Sperm recovered after cryopreservation:

- with undamaged plasma membrane 29-34%
- motile 25-36%
- progressive motile 9-13%









Semen quality before and after freezing/thawing in turkey breeds

| Semen parameters | Breeds | | |
|----------------------|------------------|----------------------|------------------|
| (%) | Romagnolo | Bronzato | Ermellinato RO |
| | | Fresh semen | |
| Membrane integrity | 95.90 ± 1.03 | 93.40 ± 1.42 | 88.73 ± 3.31 |
| Total motility | 76.64 ± 6.24 | 76.20 ± 8.10 | 73.81 ± 0.52 |
| Progressive motility | 21.47 ± 7.04 | 22.31 ± 7.23 | 15.35 ± 1.34 |
| | | Frozen/ thawed semen | |
| Membrane integrity | 51.50 ± 7.40 | 44.33 ± 1.42 | 37.38 ± 4.46 |
| Total motility | 22.38 ± 0.10 | 23.72 ± 3.10 | 14.64 ± 1.28 |
| Progressive motility | 2.57 ± 1.38 | 1.88 ± 0.68 | 0.89 ± 0.31 |

Sperm recovered after cryopreservation:

- with undamaged plasma membrane 42-54 %
- motile 20-31 %
- progressive motile 6–12 %







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Working plan 2022-2024

Breeds planned for semen storage:

- 1. Pepoi
- 2. Ermellinata di Rovigo
- 3. Robusta maculata
- 4. Robusta lionata
- 5. Livorno
- 6. Ancona
- 7. Mugellese
- 8. Valdarnese bianca
- 9. Brianzolo
- 10. Nero Italia
- 11. Tacchino di PR e PC







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Research group

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Thank you for your attention

