

Semen cryopreservation for ex situ management of genetic diversity in chicken: creation of the Italian Avian Cryobank



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INTRODUCTION

- The project “Conservation of Biodiversity in Italian Poultry Breeds – TuBAvI” (www.pollitaliani.it), MIPAAFT PSRN 2017-2020, was dedicated to the safeguard, conservation and improvement of Italian poultry genetic resources.
- A major project goal was the planning and implementation of the first Italian Semen Cryobank of Autochthonous Chicken and Turkey Breeds
- The creation of genetic resources cryobanks provides a crucial link between *in situ* and *ex situ* techniques to improve the efficiency of conservation programs.



Fig. 1. Bionda Piemontese

AIM

Implementation of the Semen Italian Cryobank of Local Poultry Breeds through the storage of semen doses of Italian chicken breeds



Fig. 2. Bianca di Saluzzo

MATERIALS and METHODS

- 25 Bionda Piemontese (BP) (Fig.1) and 18 Bianca di Saluzzo (BS) (Fig. 2) roosters were housed at the Poultry Unit, Animal Production Centre, University of Milan (Lodi, Italy).
- Semen samples were collected by abdominal massage (Fig. 3). Semen volume and concentration were recorded.
- Semen was processed for cryopreservation according to the reference procedure previously studied. In brief, semen was refrigerated at 5° C and diluted to 1 × 10⁹ sperm/mL with Lake pre-freezing medium containing 2% N-methylacetamide final concentration, loaded into 0.25 mL French straws and frozen for 10 min over a nitrogen bath at 3 cm of height. Straws were thawed at 5° C for 100 s.
- Semen was processed in many days of semen collection to start up the cryobank.
- Each day of semen collection, semen quality was assessed before and after freezing/thawing. Sperm viability (SYBR-14/ propidium iodide) (Fig. 4), motility and kinetic parameters (Sperm Class Analyzer software) (Fig. 5) were measured.
- Analysis of variance was performed to study the effect of breed and cryopreservation on semen quality.

Table 1 – Semen production in Bionda Piemontese e Bianca di Saluzzo chicken breeds.

Breed	Volume (mL)	Concentration (10 ⁹ sperm /mL)
Bionda Piemontese	0,25 ± 0,17	3,77 ± 0,76
Bianca di Saluzzo	0,33 ± 0,11	2,89 ± 0,62



Fig. 3. Semen collection

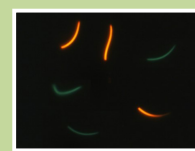


Fig. 4. Sperm viability

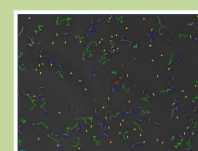


Fig. 5. Sperm motility

RESULTS and DISCUSSION

- 7 BP and 6 BS roosters were selected as donors according to their semen production and quality.
- Quantitative characteristics of the ejaculates were similar in both breeds and almost 1x10⁹ sperm were collected per ejaculate (Table 1).
- Quality of fresh semen was higher in BP compared to BS males. Sperm viability and motility were significantly higher in BP semen and also the kinetic parameters VCL, VAP and STR were significantly higher in BP semen (Table 2).
- As expected, a general significant decrease in sperm quality occurred after the freezing-thawing process and sperm quality was very similar in both breeds after thawing (Table 2).
- The Italian Semen Cryobank was started: 32 and 67 doses of frozen semen were stored in liquid nitrogen in BS and BP breeds respectively.

Table 2 – Sperm quality parameters (LSMeans ± S.E.) measured in fresh and cryopreserved semen of Bionda Piemontese (BP) and Bianca di Saluzzo (BS) cockerels.

Sperm parameters	BP BREED		BS BREED	
	Fresh semen	Cryopreserved semen	Fresh semen	Cryopreserved semen
Viability (%)	84,2 ± 9,3a	17,2 ± 6,6c	49,0 ± 9,3b	15,5 ± 6,6c
Motility (%)	86,8 ± 8,8a	17 ± 6,2c	45,1 ± 8,8b	17,4 ± 6,2c
Progressive motility (%)	13,4 ± 2,8a	1,5 ± 2b	9,1 ± 2,8a	0,8 ± 2b
VCL (µm/s)	57,3 ± 6,3a	35,2 ± 4,4b	35,5 ± 6,3b	33,1 ± 4,4b
VSL (µm/s)	19,8 ± 2,2a	10,8 ± 1,6b	14,4 ± 2,2ab	9,8 ± 1,6b
VAP (µm/s)	34,1 ± 3,8a	18,8 ± 2,7b	21,6 ± 3,8b	17,1 ± 2,7b
LIN (%)	35,0 ± 2,2ab	30,8 ± 1,6a	40,6 ± 2,2b	29,4 ± 1,6a
STR (%)	58,9 ± 2,3a	57,8 ± 1,6a	66,3 ± 2,3b	57 ± 1,6a
WOB (%)	9,3 ± 1,9a	53,3 ± 1,4b	61,0 ± 1,9a	51,4 ± 1,4b
ALH (µm)	3,7 ± 0,3	3,5 ± 0,2	2,9 ± 0,3	3,4 ± 0,2
BCF (Hz)	6,1 ± 0,9	4,1 ± 0,6	4,7 ± 0,9	3,9 ± 0,6

CONCLUSION

The present data provide basic information on sperm production, quality and sensitivity to semen cryopreservation in Italian chicken breeds; the information will improve the management of male breeder semen donors. A program to store semen doses from several Italian poultry breeds was planned and will be implemented from 2021 to 2023.

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