UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

MINISTERO DELL'AGRICOLTURA DELLA SOVRANITÀ ALIMENTARE E DELLE FORESTE

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

TUTELA BIODIVERSITÀ AVICOLA ITALIANA

# Variability in semen freezability within an Italian chicken breed

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Session 01 Chicken biodiversity
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# INTRODUCTION

- In Italy, 53 local chicken breeds were described; the majority (67%) were classified as extinct and 21% at risk of extinction.
- Attempts for conservation of Italian avian breeds are urgently required.
- Sperm cryopreservation is a powerful tool to implement the *ex situ in vitro* conservation technique in bird populations at risk

# AIM

- The aim of this study was to assess the variability in semen freezability between birds within the Italian endangered *Bianca di Saluzzo* (BS) poultry breed, in order to develop a conservation programme. Stored doses implemented the Italian Semen Cryobank for Autochthonous Poultry Breeds, born in TuBAvI project /2018-2024).

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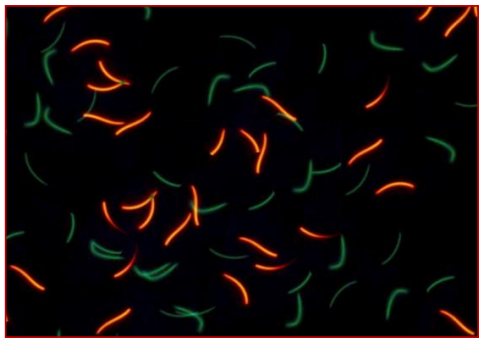
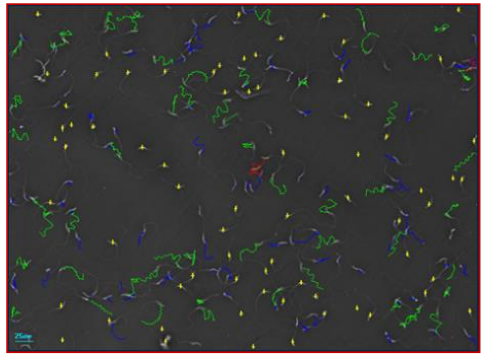
# MATERIALS and METHODS

- BS (n=18) roosters were housed at the Poultry Unit, Animal Production Centre, University of Milan (Lodi, Italy). After few weeks of acclimatization, birds were trained to semen collection and then routinely collected twice weekly for two months.



- Each day of collection were measured:
  - volume
  - concentration
  - viability (SYBR-14 and Propidium iodide)
  - total motility, progressive motility and kinetic parameters (Sperm Class Analyzer (SCA) software)



- Among the roosters that responded to manual semen collection, only subjects that produced ejaculates with sperm viability < 70% and total motile sperm < 65% were selected.

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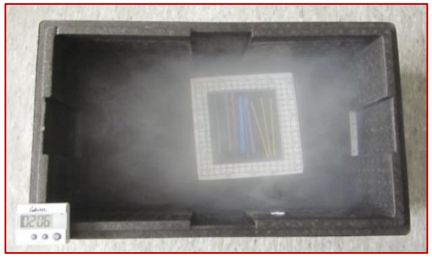
# MATERIALS and METHODS

- Semen, after a cooling step at 4°C for 20min, was finally diluted to $1\times10^9$ sperm/mL with Lake pre-freezing medium containing 2% N-methylacetamide, loaded into 0.25 mL French straws, frozen for 10 min over a nitrogen bath at 3 cm of height and stored in liquid nitrogen at -196 ° C in cryogenic tank (Iaffaldano et al., 2021).

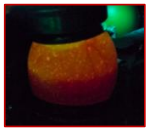
- The straws were thawed at 5°C for 100 s and *in vitro* sperm quality parameters were analyzed as in fresh semen



## *In vivo* trial

- The donors with the highest number of semen doses were chosen for the *in vivo* trial in order not to affect the cryobank sperm doses reserve

- A total of 10 laying hens per selected male donors were inseminated twice (day 1 and 3) to assess *in vivo* fertility and embryo viability (by candling after 7 days of incubation).



1 straw = $250\text{x}10^6$ sperm

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# RESULTS and DISCUSSION

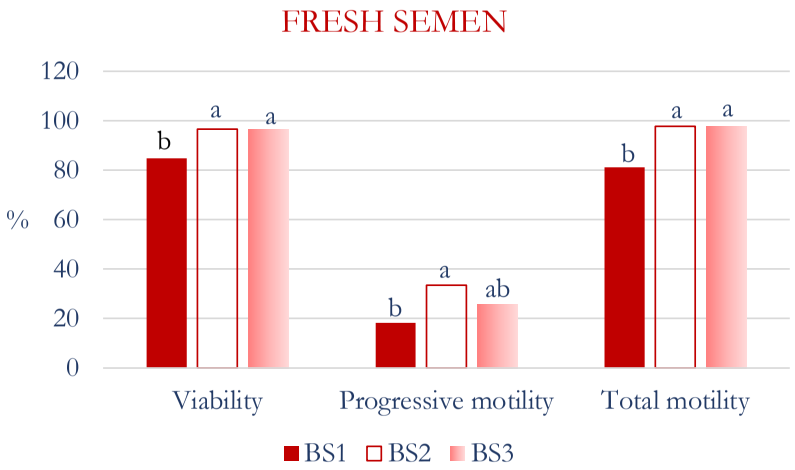
- The mean volume and sperm concentration recorded in fresh ejaculates were 0.327±0.17 mL and 3.303±1.27 x $10^9$ sperm/mL

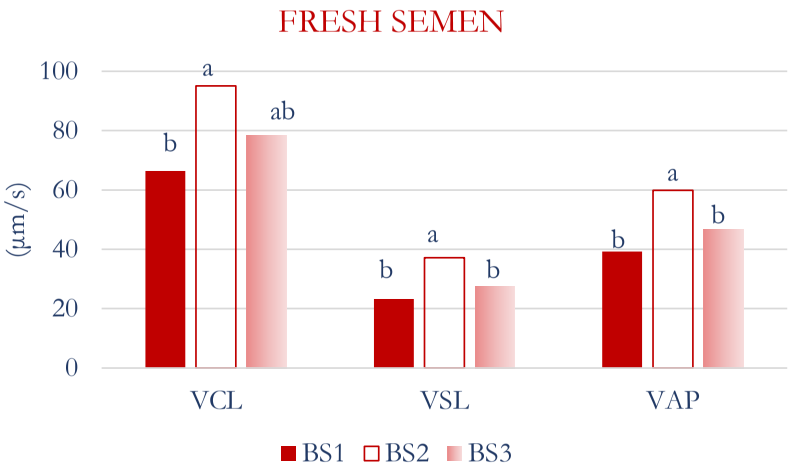
| Animals | Viability (%) | Total motility (%) | Progres motility (%) | VCL (µm/s) | VSL (µm/s) | VAP (µm/s) | LIN (%) | STR (%) | WOB (%) | ALH (µm) | BCF (Hz) | paillettes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BS1 | 84.7b | 81.1 | 18.3b | 66.4b | 23.2b | 39.2b | 35.4 | 59.9 | 59.0 | 3.8 | 6.6 | 64 |
| BS2 | 96.6a | 97.7 | 33.4a | 95.0a | 37.1a | 59.9a | 39.4 | 62.2 | 63.1 | 4.1 | 7.4 | 38 |
| BS3 | 96.4a | 97.7 | 25.7ab | 78.3ab | 27.3b | 46.6b | 35.1 | 58.7 | 59.6 | 3.9 | 7.2 | 37 |
| BS4 | 88.3ab | 89.6 | 22.0b | 72.6b | 27.1b | 45.3b | 37.5 | 60.0 | 62.5 | 3.9 | 6.4 | 25 |
| BS5 | 94.1ab | 96.2 | 30.3a | 88.7ab | 34.2a | 56.1a | 39.6 | 61.4 | 64.0 | 4.2 | 7.1 | 25 |
| BS6 | 86.7b | 83.3 | 19.2b | 61.6b | 23.1b | 37.6b | 37.8 | 61.6 | 61.0 | 3.6 | 6.8 | 17 |

- Only 6 were selected as donors and 3 birds (BS1, BS2, BS3) were used for artificial insemination trial, according to availability of semen doses.

# RESULTS and DISCUSSION

## Sperm quality variables in fresh semen



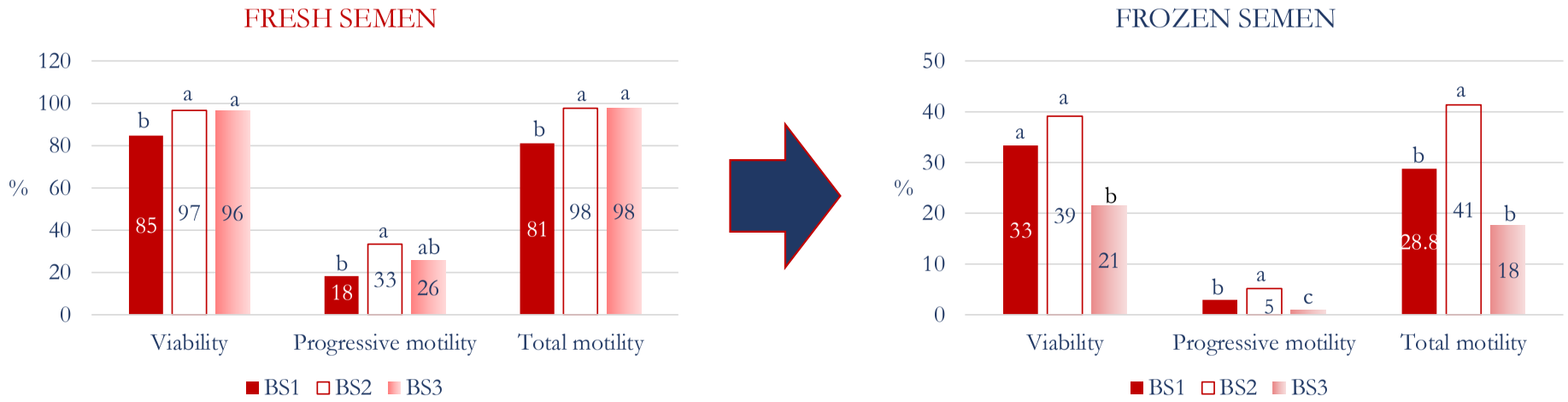

Stats: PROC GLM SAS software

VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity

# RESULTS and DISCUSSION

## Sperm quality variables in frozen/thawed semen

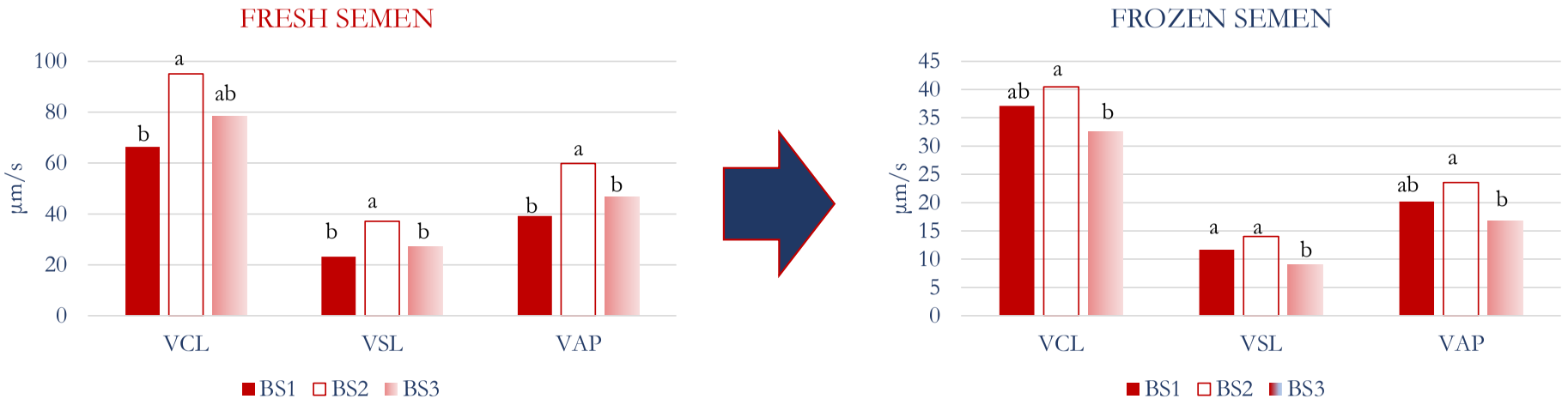
- As expected, a general significant decrease in sperm quality occurred after the freezing-thawing process.




Stats: PROC MIXED SAS software

# RESULTS and DISCUSSION

## Sperm quality variables in frozen/thawed semen

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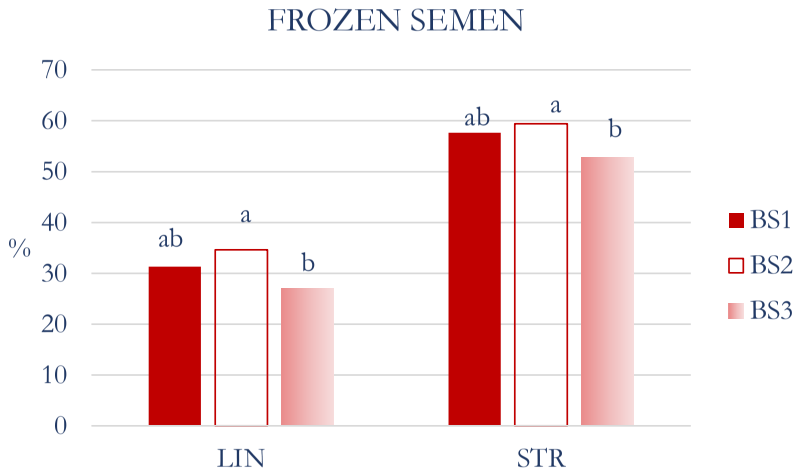
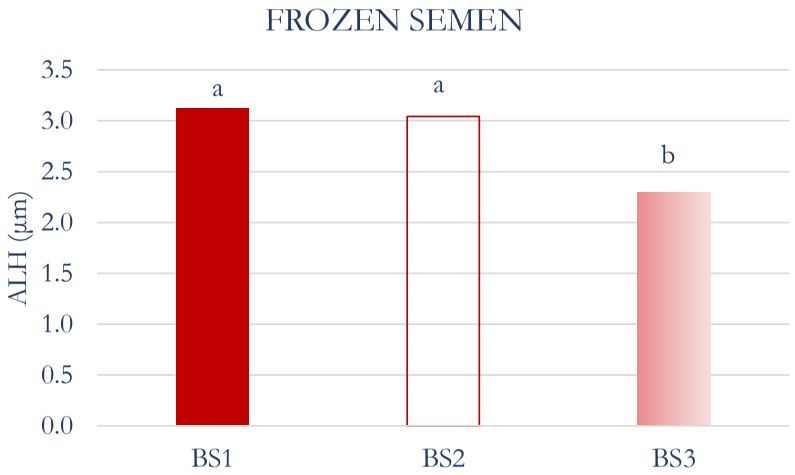
# RESULTS and DISCUSSION

## Sperm quality variables in frozen/thawed semen



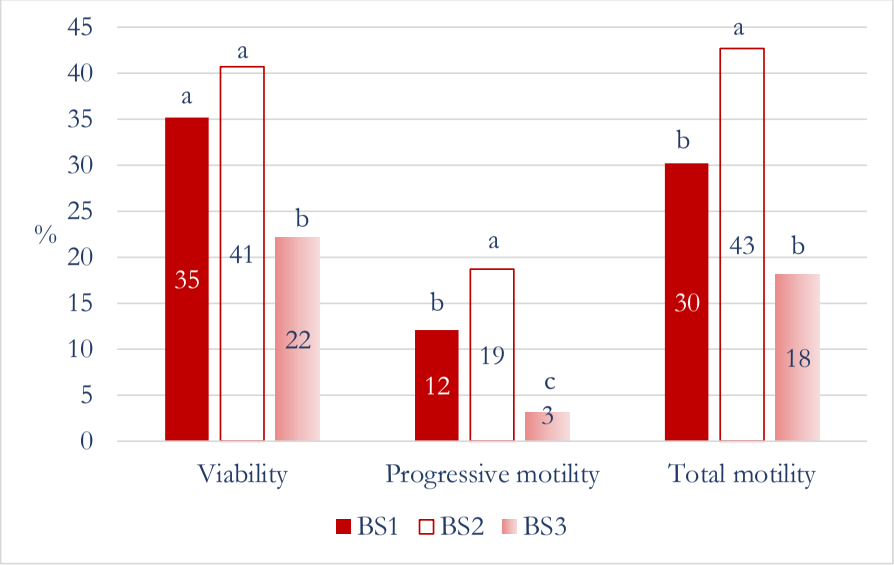




LIN: linearity (VSL/VCL x 100); STR: straightness (VSL/VAP x 100)

ALH: amplitude of lateral head displacement

# RESULTS and DISCUSSION

## Recovery rates

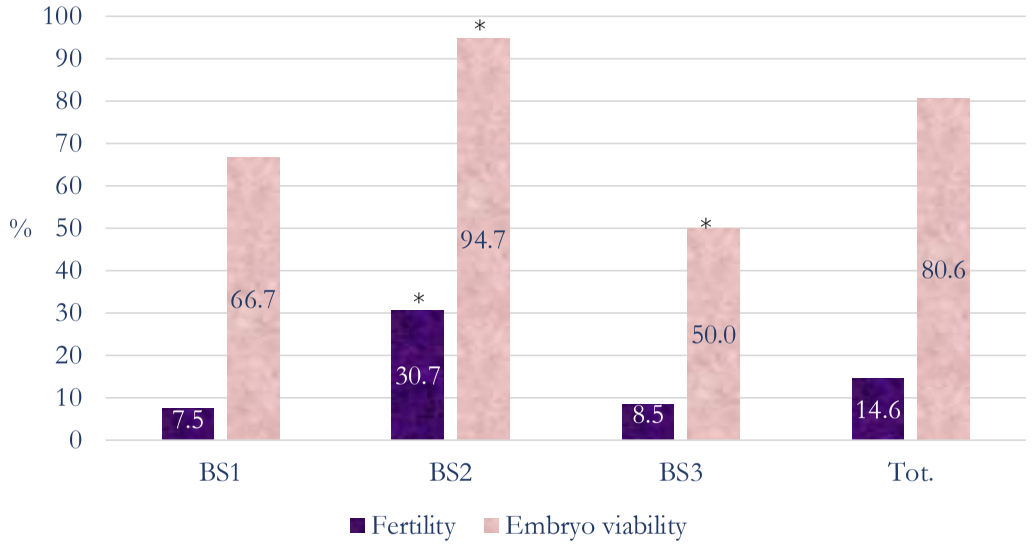



$$\text{Recovery} = \frac{\text{mean value thawed semen}}{\text{mean value fresh semen}} \times 100$$

Stats: PROC GLM SAS software

# RESULTS and DISCUSSION

## Fertility and embryo viability




Stats: Chi-square test for *in vivo* data

# CONCLUSIONS

- High individual variability was found in semen freezability, not always related to fresh sperm quality.
- *In vitro* quality of thawed semen was associated to the results obtained in the artificial insemination test
- Furthered analyzes must be performed to confirm the present results and to identify quality sperm markers for the selection of the best semen donors in conservation programs.

# Thank you for your attention!

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