ASPA 2019 SORRENTO

23° Congress of the Animal Science and Production Association

NEW CHALLENGES IN ANIMAL SCIENCE

June 11-14, 2019 – Sorrento (Italy)

How can the honey improve the post-thaw quality of turkey spermatozoa?

Michele Di Iorio, Giusy Rusco, Angelo Manchisi, Silvia Cerolini, Nicolaia Iaffaldano

Loss of biodiversity

Extensive breeding

populations

native breeds

Intensive breeding

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

Progetto dedicato alla salvaguardia, conservazione e valorizzazione del patrimonio genetico avicolo italiano che nasce dall'associazione degli Atenei italiani impegnati da anni nella conservazione di biodiversità in avicoltura attraverso il mantenimento diretto di popolazioni di razze autoctone.

TUTELA DELLA BIODIVERSITÀ NELLE RAZZE AVICOLE ITALIANE – TuBAvI

Progetto collettivo beneficiario per il Comparto Avicoli con il sostegno del Fondo Europeo Agricolo per lo Sviluppo Rurale (FEASR)
https://ec.europa.eu/agriculture/rural-development-2014-2020_it

MIPAAFT – Programma Sviluppo Rurale Nazionale 2014/2020
Sottomisura 10.2 – Sostegno per la conservazione, l'uso e lo sviluppo sostenibile delle risorse genetiche in agricoltura.

UNIVERSITÀ DI PISA

Conservation *ex situ in vitro*



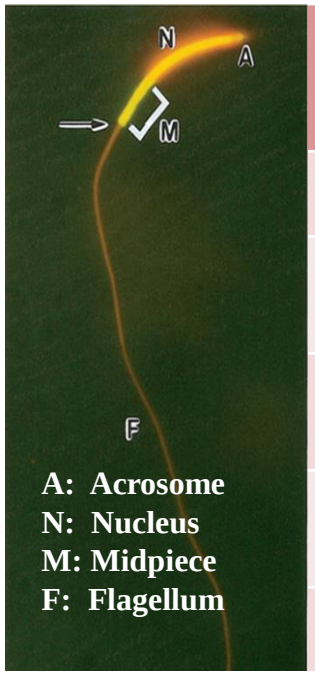


Semen cryopreservation is the only reproductive technology which is currently available for **birds** because of the **inability to freeze embryos and oocytes**

TUTELA BIODIVERSITÀ AVICOLA ITALIANA

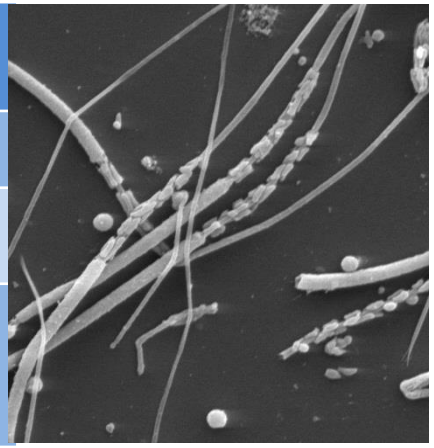
1st Italian Semen Cryobank of Autochthonous Poultry Breeds

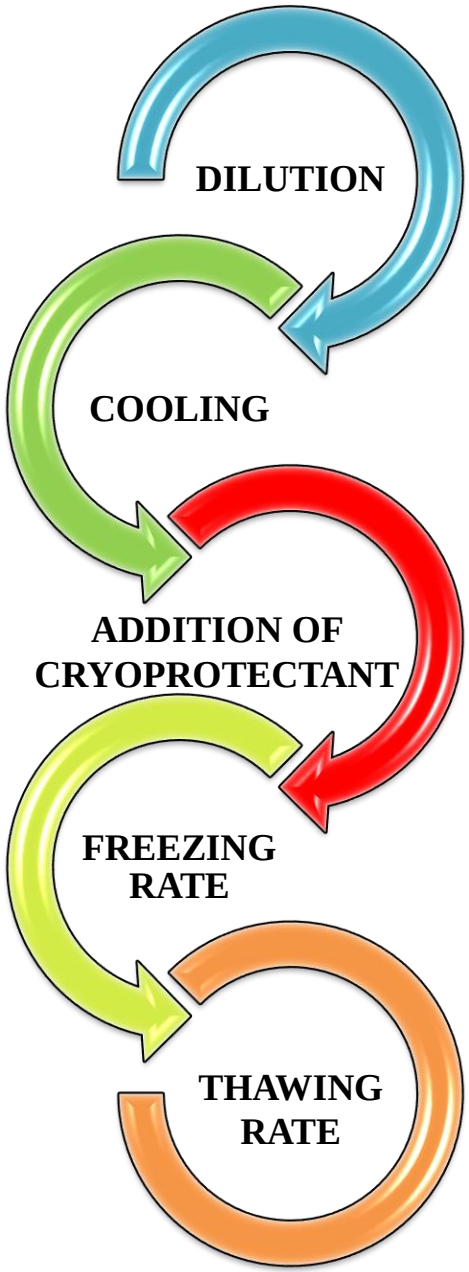
Cryopreservation of avian semen



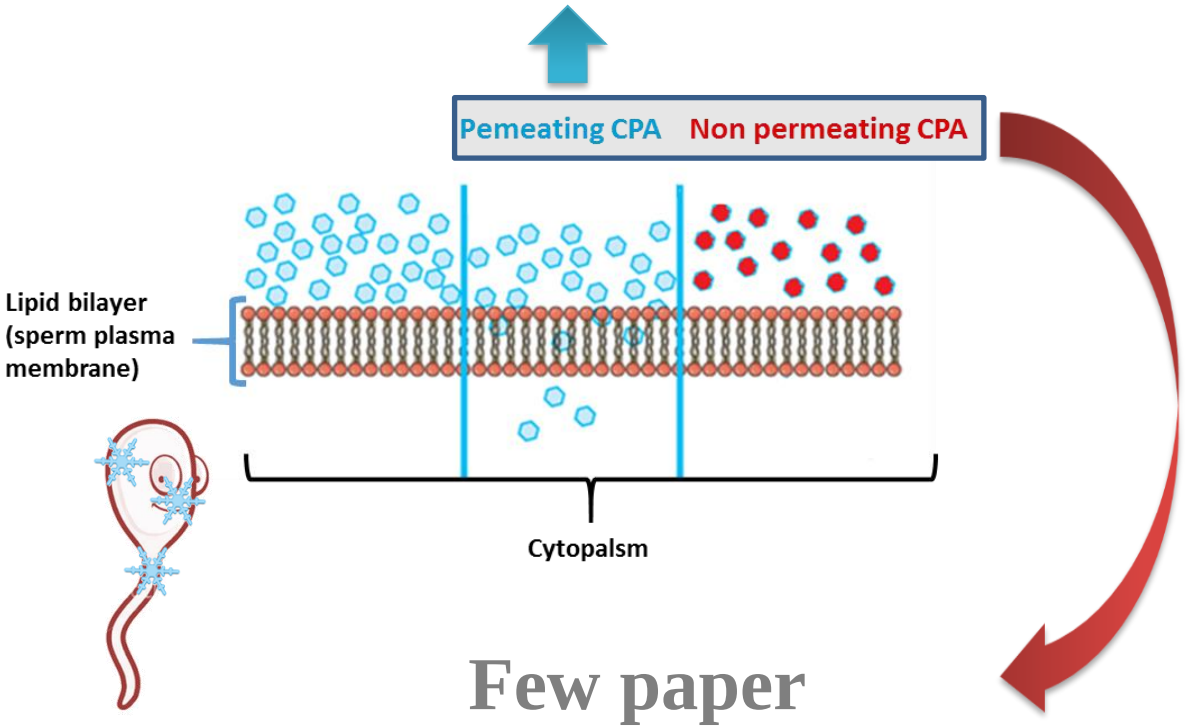
Major factors causing low turkey sperm cryosurvival	References
Filiform shape, long tail and condensed nucleus	Long, 2006
High cholesterol /Phospholipid ratio of membrane	Blesbois et al., 2005
Low membrane permeability	Blanco et al., 2000, 2008
Low osmotolerance to hypertonicity	Blanco et al., 2008, 2011
Low membrane fluidity	Blesbois et al., 2005

Main cryoinjuries in turkey sperm cells	References
Loss of sperm ATP	Wishart and Palmer, 1986; Blanco et al., 2011
Irreversible damage to sperm mitochondria and nucleus	Bakst and Sexton, 1979; Long, 2006
Loss of motility and plasmamembrane structural integrity	Blesbois et al., 2005 Blanco et al., 2000, 2008, 2011; Cerolini et al., 2008; Iaffaldano et al., 2011





CRYOPROTECTANT	REFERENCES
DMA	Blanco et al., 2000 Iaffaldano et al., 2011 Long et al., 2014
DMSO	Sexton 1979 Iaffaldano et al., 2016
Glycerol	Pandian et al., 2011 Long et al., 2014



Few paper

Aim

- **Trehalose**
- **Sucrose**
- **Ficoll**

\+ **DMSO**

HONEY

References


Asian Pacific Journal of Reproduction

Natural honey as a cryoprotectant to improve Arab stallion post-thawing sperm parameters


Exploration of natural cryoprotectants for cryopreservation of African catfish, *Clarias gariepinus*, Burchell 1822 (Pisces: Clariidae) spermatozoa


Research Article

Cryopreservative Effect of Adding a Honey Solution to Native Chicken Spermatozoa

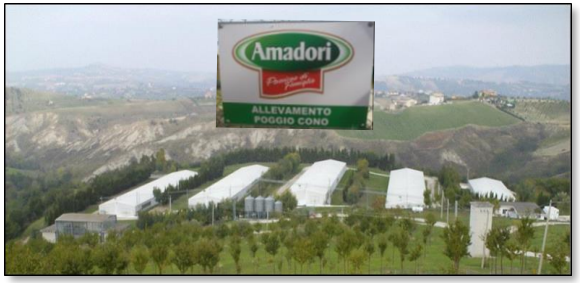

Honey Supplementation to Semen-Freezing Medium ImproveHuman Sperm Parameters Post-Thawing

The goal of this study was to investigate the effect of different concentration of **bee honey** as non-permeant cryoprotectant on the **cryosurvival of turkey semen**

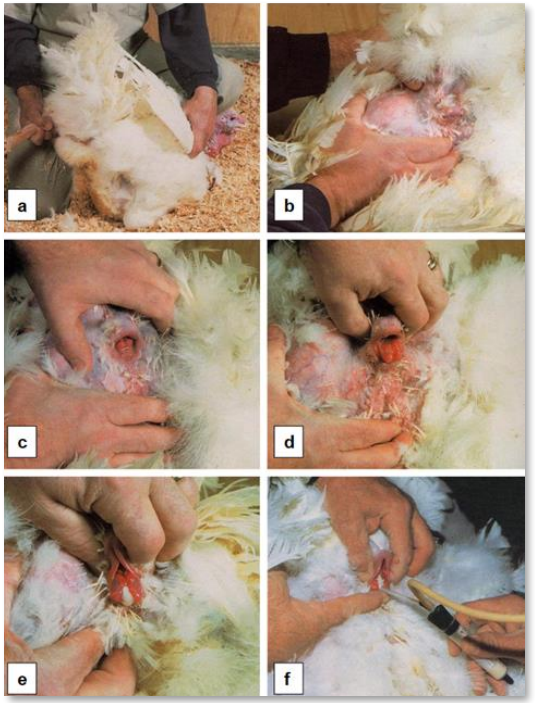
Materials and Methods

1. Animals

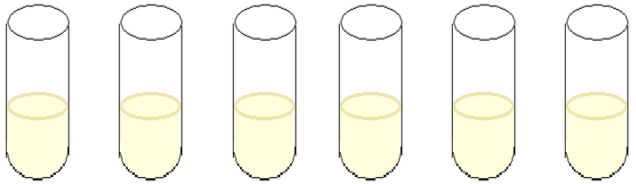
40 ♂



2. Semen collection

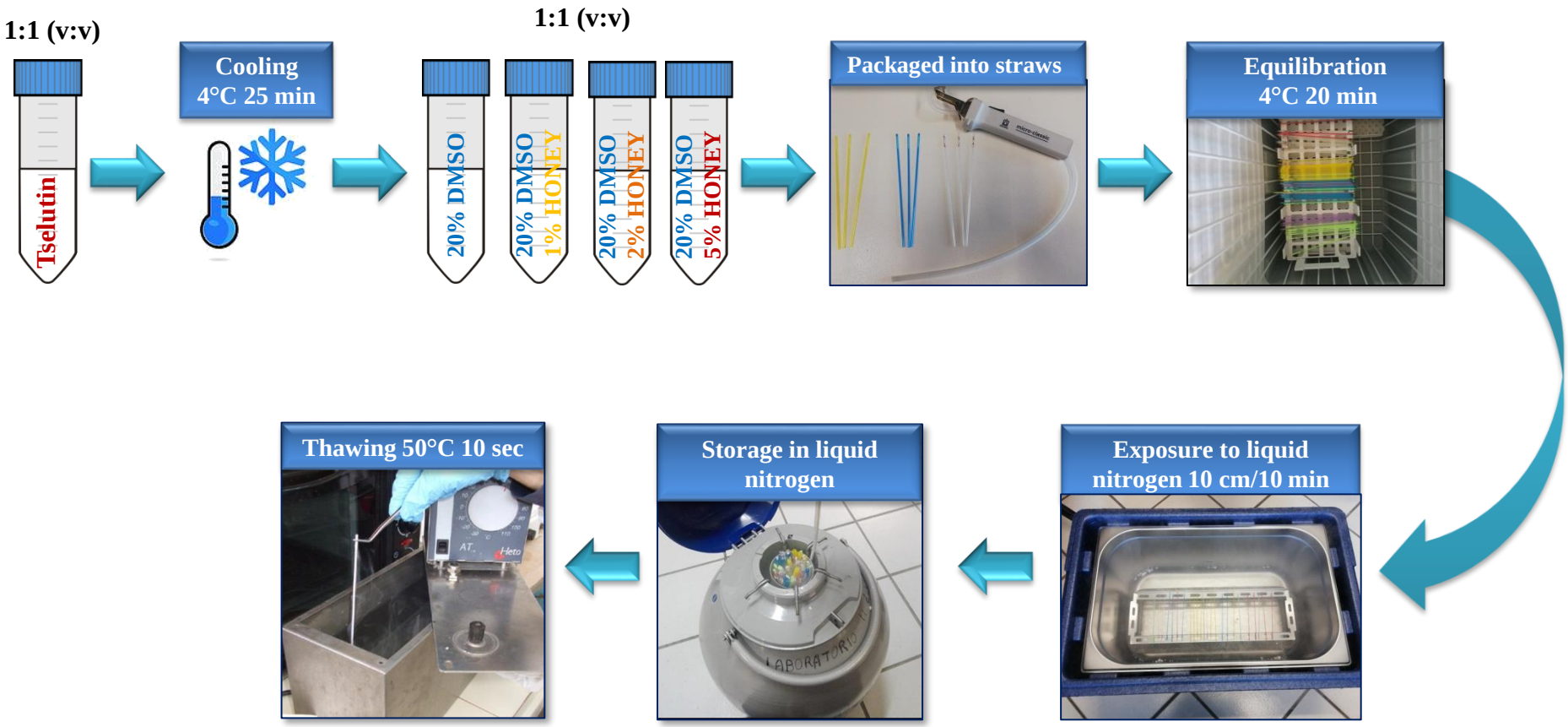


6 POOLS



Materials and Methods

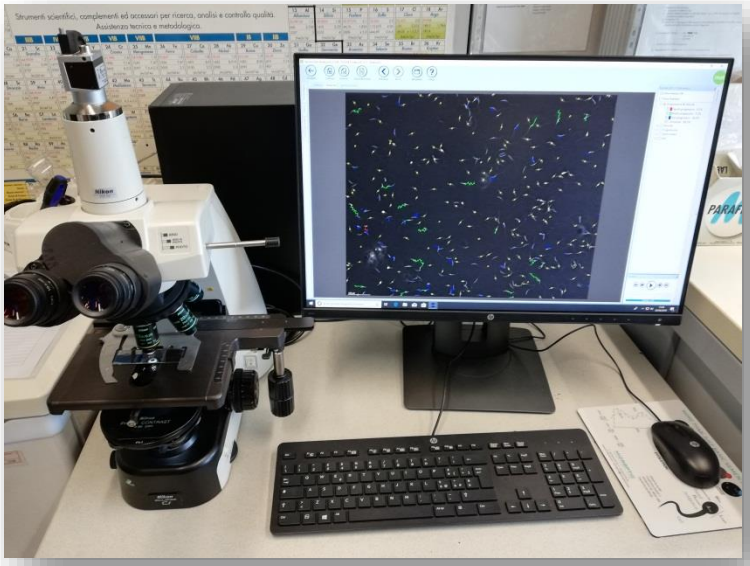
3. Freezing protocol



Materials and Methods

4. Sperm quality - Motility

CASA-system
software Sperm Class Analyzer-SCA
Nikon mod. Ci-L



1 Dilution
100×10^6 spz/mL

2 Incubation
38°C 5 min

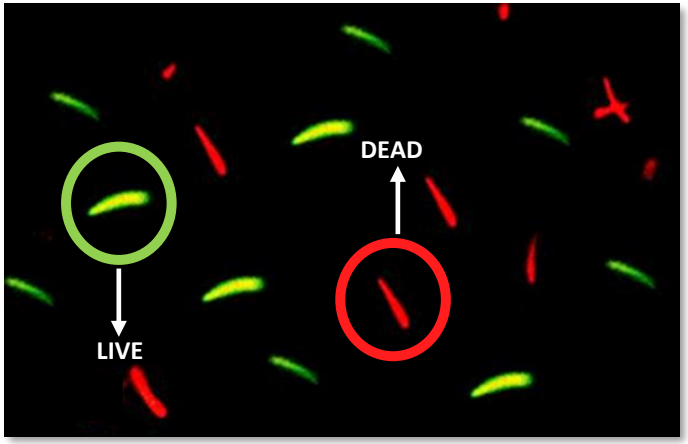
- **Total Motility (%)**
- **Progressive Motility (%)**
- **VCL (µm/s)**
- **VSL (µm/s)**
- **VAP (µm/s)**
- **LIN (%)**
- **STR (%)**

Materials and Methods

4. Sperm quality - Viability and Osmotic Resistance

LIVE/DEAD Sperm Viability kit

- **5 µL of semen**
- **80 µL Tselutin** → **Viability**
  80 µL H_2O → **Osmotic resistance**
- **2 µL SYBR-14** (10 min/38°C/dark)
- **5 µL PI** (5 min/38°C/dark)
- **examined 100× oil immersion** objective under epifluorescence illumination



% viability
osmotic resistance

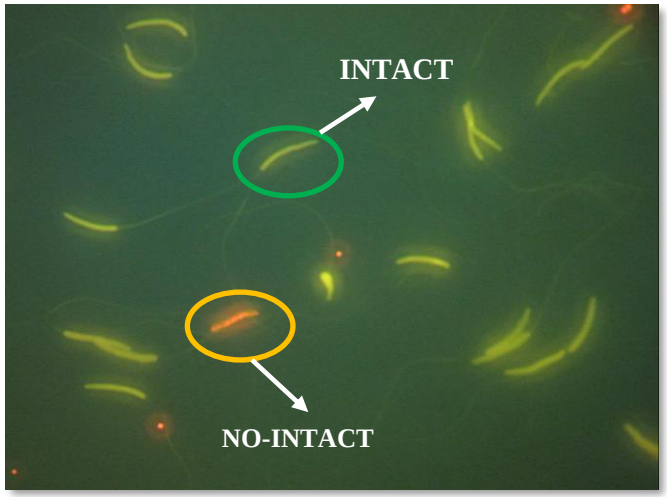
$$\frac{\text{Green spermatozoa}}{\text{Green spermatozoa} + \text{Red spermatozoa}} \times 100$$

Materials and Methods

4. Sperm quality - DNA integrity

Acridine Orange Test

- **5 µL of semen**
- **100 µL Tselutin** → **smeared** 
- fixed overnight in a **3:1 methanol:glacial acetic acid** 
- stained with **AO solution** (0.2 mg/mL in water)
- **incubation** (5 min/room temperature/dark)
- examined **fluorescence microscope** (100×) with a 490 nm excitation light and 530 nm barrier filter

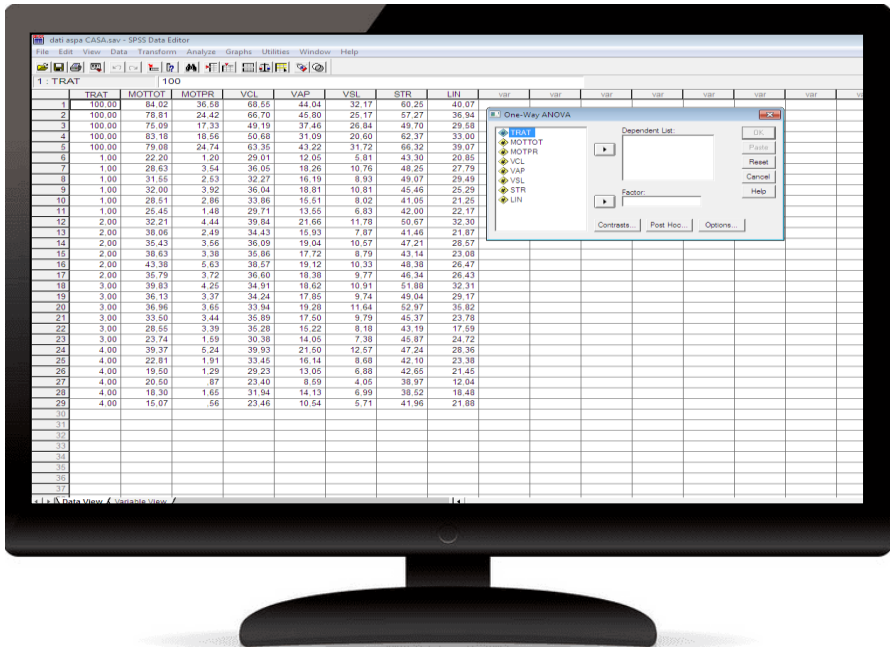


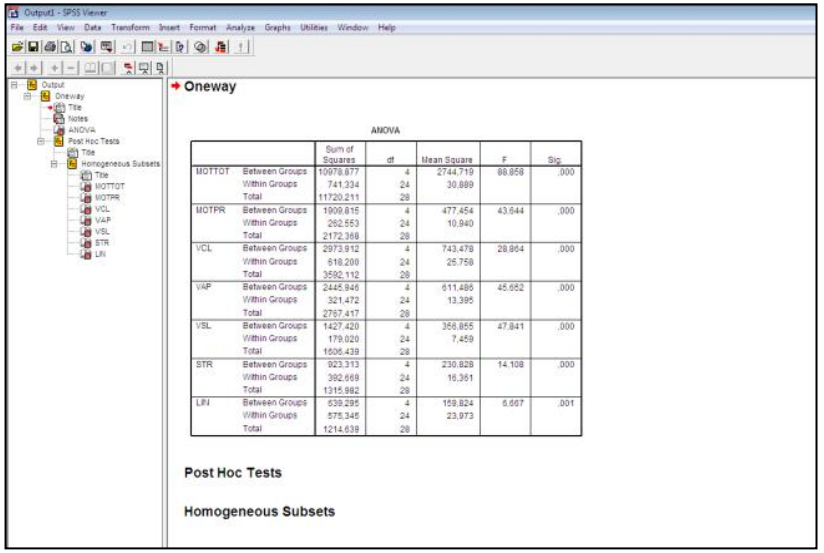
$$\%\ \text{DNA integrity} = \frac{\text{Green spermatozoa}}{\text{Green spermatozoa} + \text{Red spermatozoa}} \times 100$$

Materials and Methods

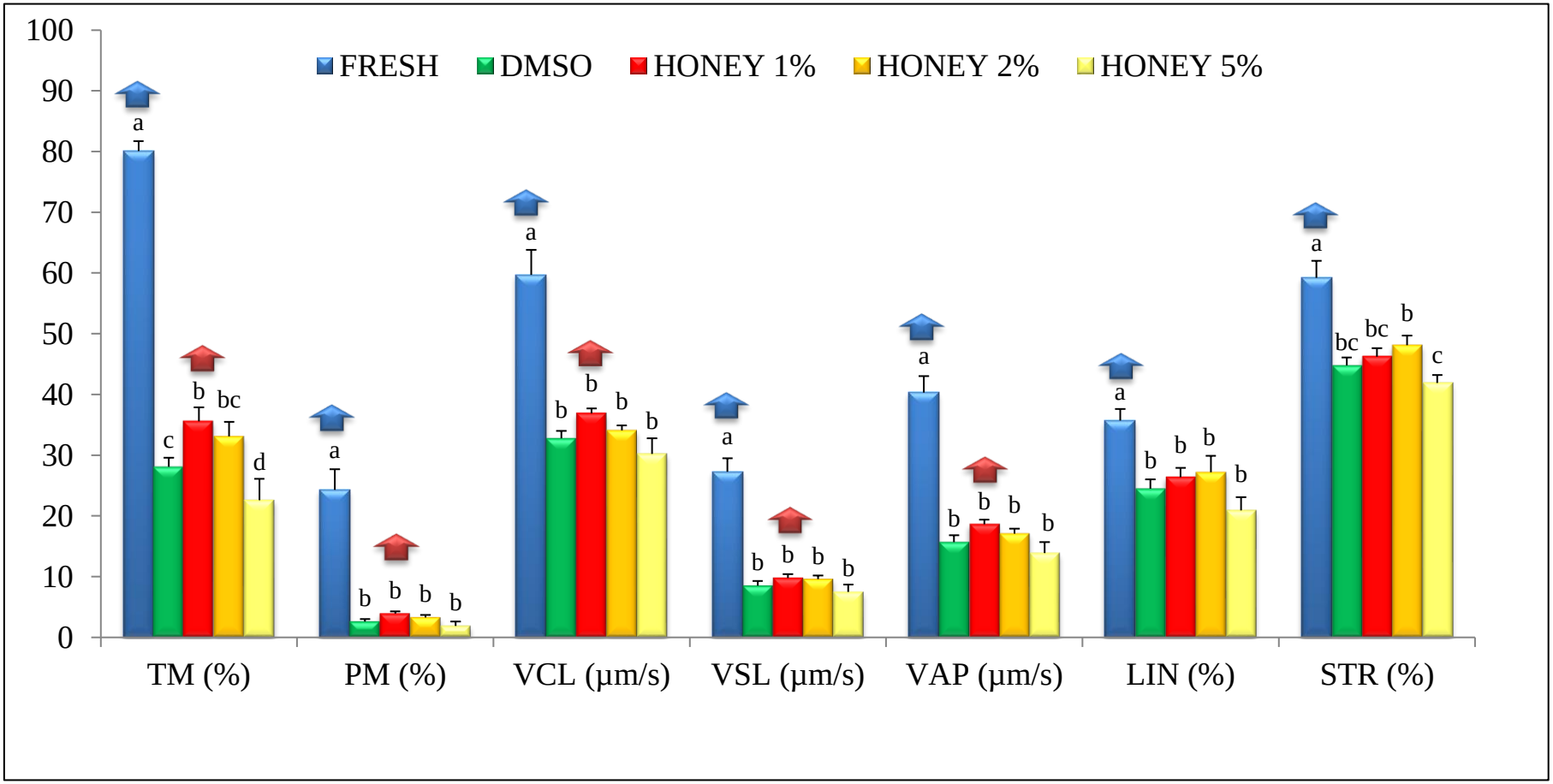
5. Statistical analysis

- Sperm quality parameters were analyzed by **ANOVA**
- **DUNCAN** comparison test
- Significance was set at **P < 0.05**



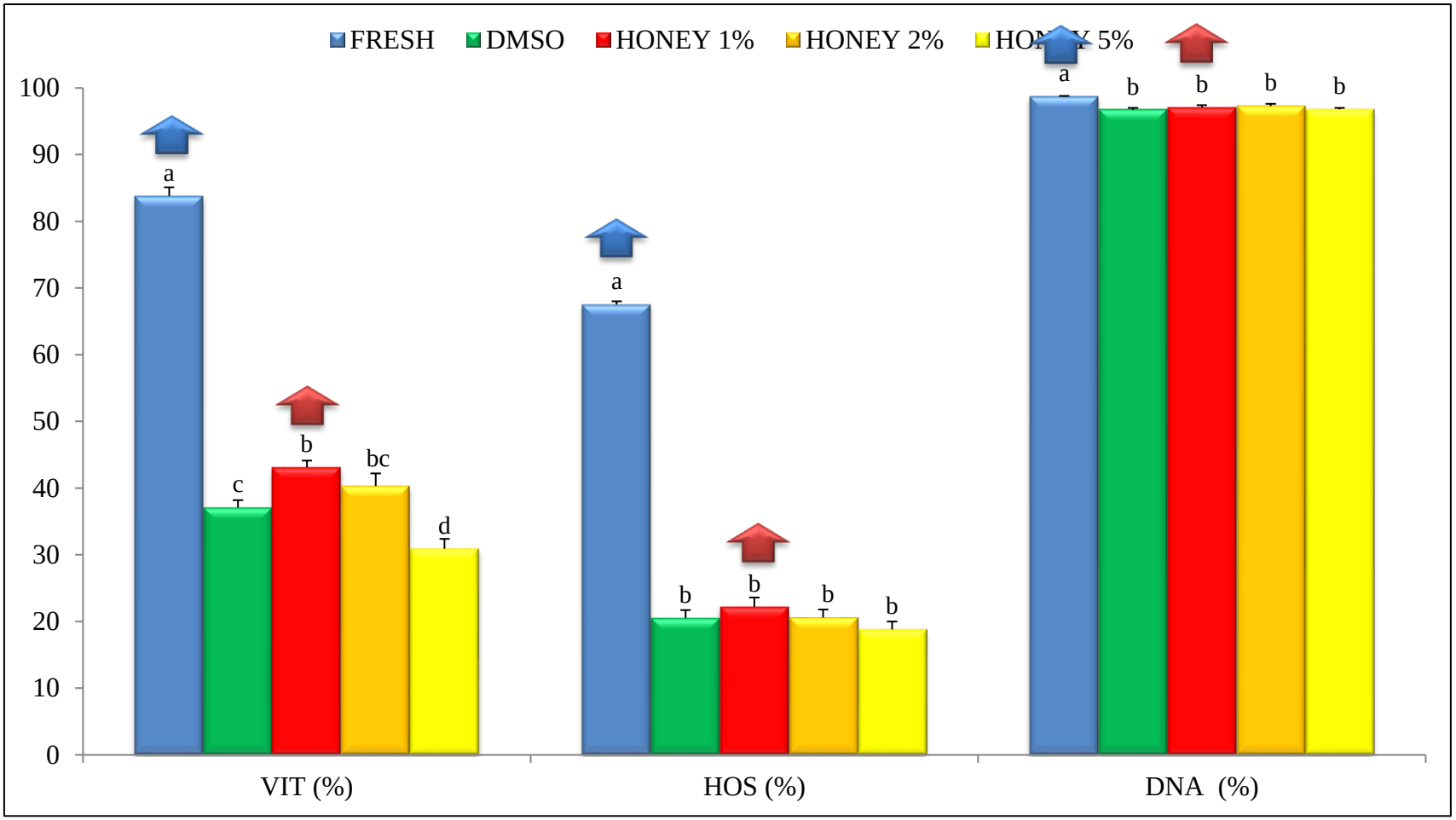


Results



TM: Total Motility; **PM:** Progressive Motility; **VCL:** Curvilinear velocity; **VSL:** straight-line velocity; **VAP:** average path velocity; **LIN:** Linearity; **STR:** Straightness

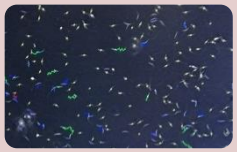
Results

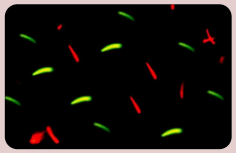


VIT: Viability; **HOS:** Osmotic Resistance; **DNA:** DNA integrity

Discussions and Conclusions

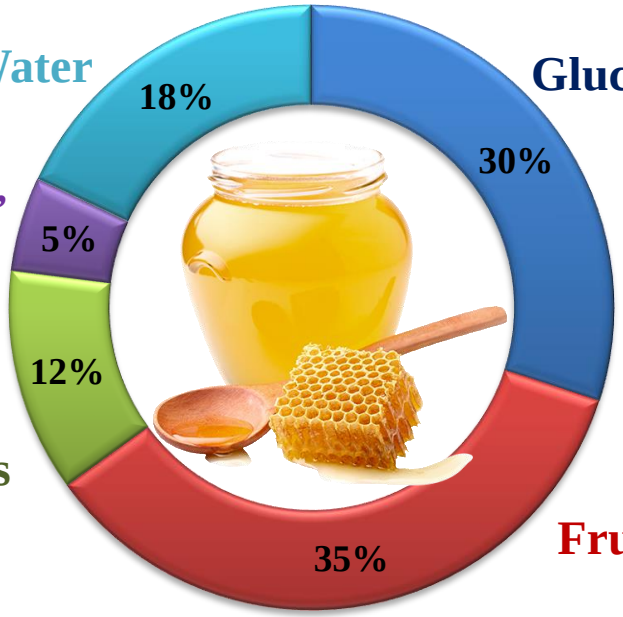
Freezing/thawing process





1%  **DMSO**

How can the honey improve the post-thaw quality of turkey spermatozoa?

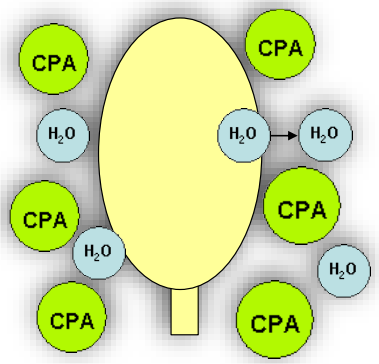


Discussions and Conclusions

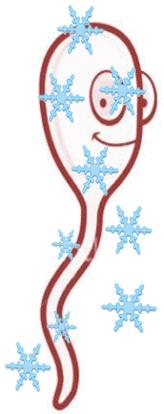
Source of energy

Gil et al., 2010; Rahardhianto et al., 2012

Adequate cellular dehydration

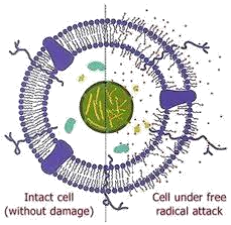


Reducing ice-crystals



Flavonoids and phenolic compounds

Reactive oxygen species ROS



El-Sheshtawy et al., 2014, 2016

Malik et al., 2019

Discussions and Conclusions

Development of an effective freezing protocol for turkey semen using honey as a natural NP-CPA for the first time

TUTELA BIODIVERSITÀ AVICOLA ITALIANA

1st Italian Semen Cryobank of Autochthonous Poultry Breeds


Thank you
for
your attention