



## 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



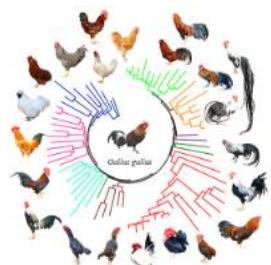
# Loss of biodiversity

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## 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

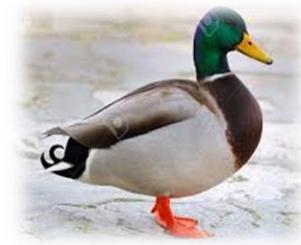
Progetto collettivo beneficiario per il  
Comparto Avicoli con il sostegno del  
Fondo Europeo Agricolo per lo Sviluppo Rurale  
(FEASR)

[https://ec.europa.eu/agriculture/rural-development-2014-2020\\_it](https://ec.europa.eu/agriculture/rural-development-2014-2020_it)

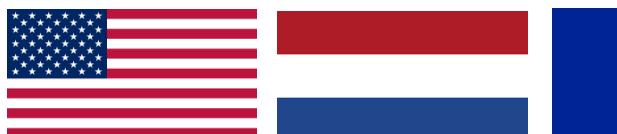
## MIPAAFT – Programma Sviluppo Rurale Nazionale 2014/2020

Sottomisura 10.2 – Sostegno per la conservazione, l'uso e lo sviluppo sostenibile delle risorse genetiche in agricoltura.

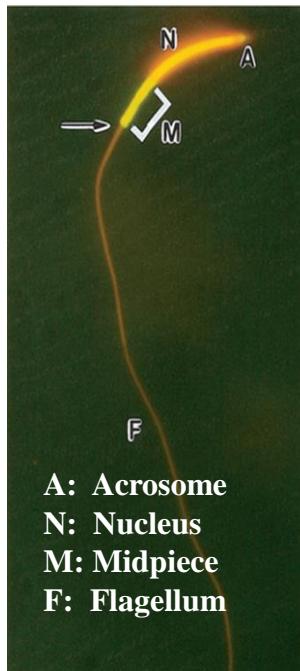
# Conservation *ex situ in vitro*



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

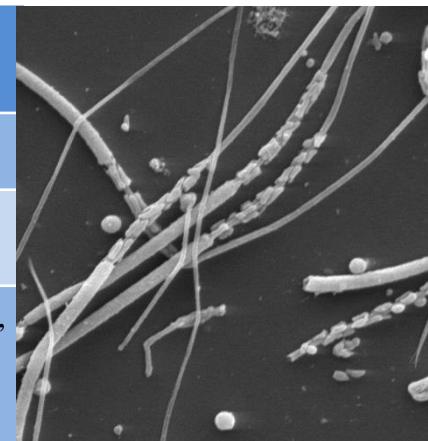


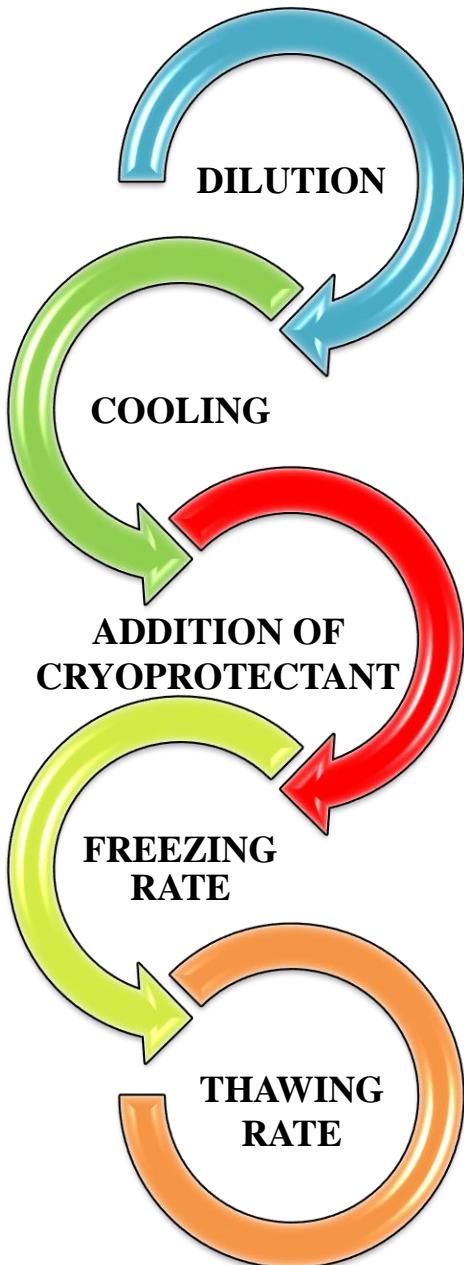
# Cryopreservation of avian semen



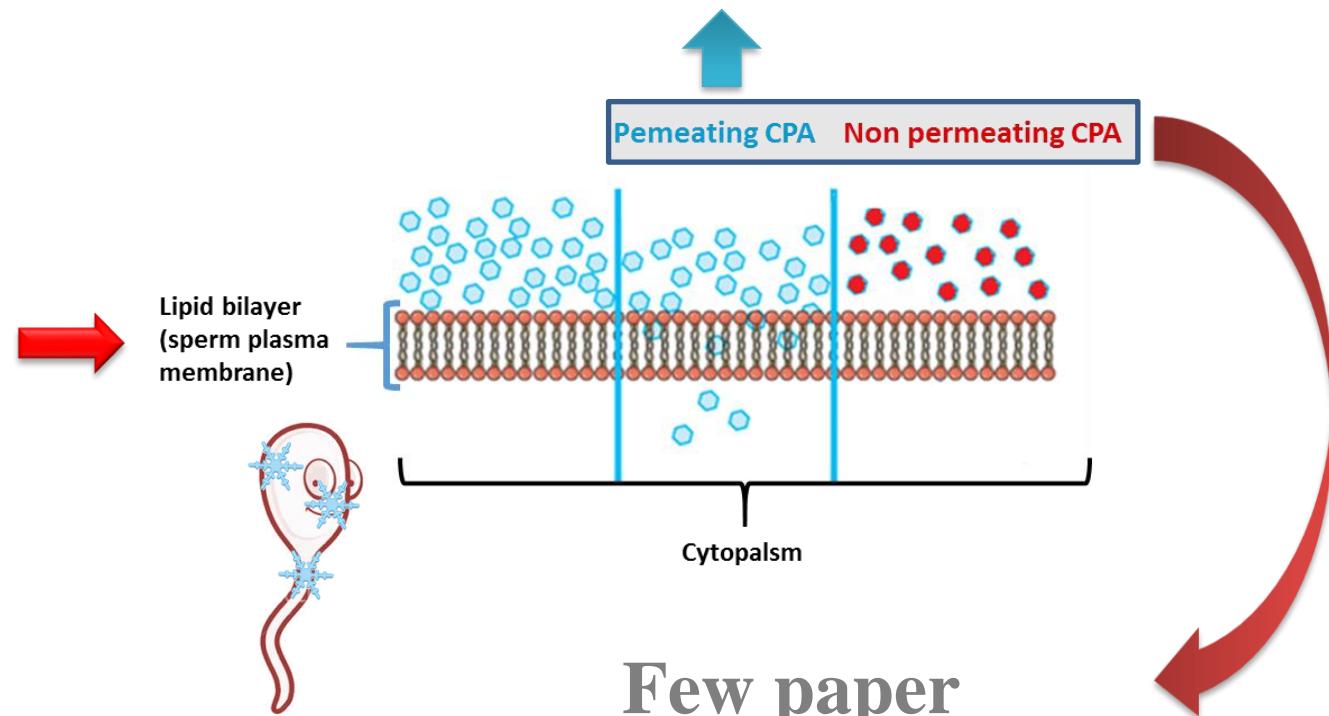
Major factors causing low turkey sperm cryosurvival	References
<b>Filiform shape, long tail and condensed nucleus</b>	Long, 2006
<b>High cholesterol /Phospholipid ratio of membrane</b>	Blesbois et al., 2005
<b>Low membrane permeability</b>	Blanco et al., 2000, 2008
<b>Low osmotolerance to hypertonicity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

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





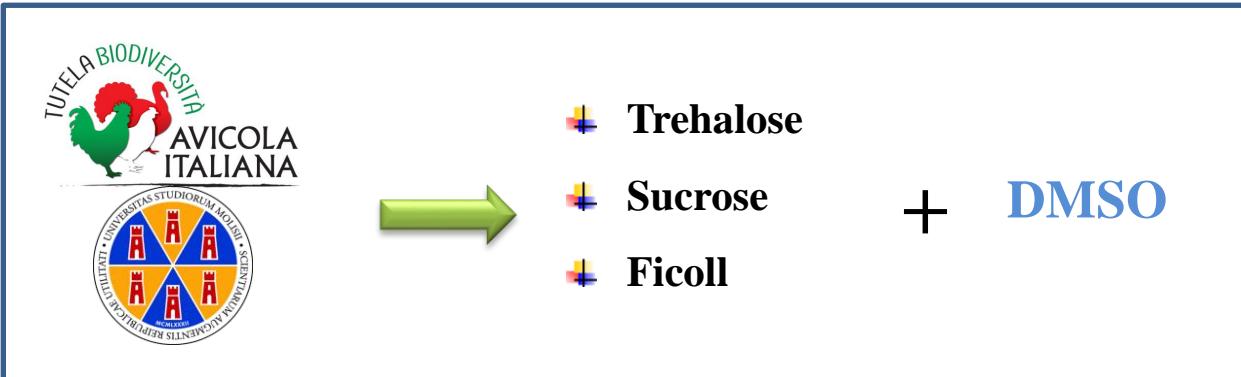
CRYOPROTECTANT	REFERENCES
DMA	Blanco et al., 2000 Iaffaldano et al., 2011 Long et al., 2014
DMSO	Sexton 1979 Iaffaldano et al., 2016
Glycerol	Pandian et al., 2011 Long et al., 2014



Few paper



# Aim



## References



**Asian Pacific Journal of Reproduction**

Volume 6 Number 4 December 7, 2011

**Original Article**

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

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**Keywords:** Sperm; Semen; Post-freezing; Honey

Original Paper  
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doi:10.7713/cjas.2010-0002

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

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**ABSTRACT** Objective: To investigate the effect of certain cryoprotectants, sucrose, dextrose, trehalose, glycerol, and sucrose monoglyceride on African catfish spermatozoa after cryopreservation at 2°C. Materials and methods: Semen of four strains of four different species of Arabian stallions were subjected to cryopreservation with a standard (INA-02), without any cryoprotectant (control), or with 1% sucrose, 1% trehalose, 1% glycerol, 1% sucrose monoglyceride, or 1% glycerol monoglyceride. Semen freezing was performed at -1, 0 and 2°C. Sperm viability was measured at 2°C, while motilities were performed at 0, 1 and 2°C. Sperm viability and motility percentages of each sample were determined by conventional laboratory methods. Results: Motility of semen frozen at 2°C was significantly higher than that frozen at 0°C and -1°C ( $P < 0.01$  at least). Post-thaw sperm motility viability reduced percentage ( $P < 0.05$ ) in both 0 and 2°C post-freezing. For all semen parameters, the control group had the lowest ( $P < 0.05$ ) post-thaw sperm motility and viability compared to the groups treated with 1% sucrose monoglyceride. Conclusion: Honey can be used as a non-permeant cryoprotectant to improve sperm quality in comparison to the control group.

**Keywords:** Honey; Native chicken; Semen qualities; Cryopreservation; Post-freezing

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Research Article  
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Advances in Animal and Veterinary Sciences

### Cryopreservative Effect of Adding a Honey Solution to Native Chicken Spermatozoa

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<sup>3</sup>Abstract: The current study was conducted to determine the quality of semen of native chicken Kalimantan breed. A total of four code was used in this study. Semen was collected twice per week via the dorsal-dorsal incision method. Semen quality was very important to the success of breeding in the chicken. The semen was determined macroscopically and microscopically. Five groups of semen were used in this study. Group 1 (G1) was control, group 2 (G2) was added with 1% of cryoprotectant solution (G3), group 3 (G4) was, or mixed with 5% honey (G5) or with 10% honey (G6). Semen was collected from the male chicken and was immediately frozen at -1°C for 24 hours. Semen was then thawed after 24 hours. Direct semen was used for in vitro semen insemination post-thawing. After insemination, the semen was then placed in a petri dish and was observed under a light microscope.

**Results/Results:** It appeared that the percentage of sperm motility for G3 and G2 groups were significantly reduced ( $P < 0.05$ ) in the total motility (% of the post-thaw sperm between the G1 and G2 groups) compared to the control group. The percentage of sperm motility viability for G3 and G4 groups were significantly reduced ( $P < 0.05$ ) in the total motility (% of the post-thaw sperm between the G1 and G3 groups) compared to the control group. The percentage of sperm motility viability for G5 and G6 groups were significantly reduced ( $P < 0.05$ ) in the total motility (% of the post-thaw sperm between the G1 and G5 groups) compared to the control group. The percentage of sperm motility viability for G6 group was significantly lower than that of G5 group. The addition of honey to the semen post-thawing did not affect the sperm quality in the semen post-thawing.

**Conclusion:** Honey has no significant reduction ( $P < 0.05$ ) in the sperm agglutination (% of G3 group) as compared to the control group.

**Keywords:** Honey; Native chicken; Semen qualities; Cryopreservation; Post-freezing

### Honey Supplementation to Semen-Freezing Medium Improves Human Sperm Parameters Post-Thawing

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Received manuscript accepted and accepted online 2011

**Abstract** The aim of evaluating the effect of honey supplementation to cryoprotective medium on post-thaw sperm motility, concentration, morphology and survival. Materials and methods: Thirty semen samples were collected from 30 different donors. After semen collection, the semen was divided into three groups (G1, G2, G3). Group 1 (G1) contained 10% glycerol (G1) + 1% sucrose (S1) + 1% trehalose (T1) + 1% honey (H1) + 1% DMSO (D1). Group 2 (G2) contained 10% glycerol (G2) + 1% sucrose (S2) + 1% trehalose (T2) + 1% honey (H2) + 1% DMSO (D2). Group 3 (G3) contained 10% glycerol (G3) + 1% sucrose (S3) + 1% trehalose (T3) + 1% honey (H3) + 1% DMSO (D3). Motility, concentration, morphology and survival of the membrane plasma between treatments and the control before freezing. However, sperm motility before freezing was significantly different ( $P=0.05$ ) between the control and all treatments. The addition of honey to the semen before freezing did not affect the sperm motility, motility, concentration and integrity of the membrane plasma were not significantly different ( $P=0.05$ ) between the control and all treatments. The motility of spermatozoa before freezing for the P4 treatment was significantly lower than that of the control group. The addition of honey to the semen post-thawing did not affect the sperm quality in the semen post-thawing.

**Conclusion:** The results of this study indicated that the supplementation of honey (10%) to cryoprotective solution results in enhancement of sperm quality post-thawing.

**Keywords:** Honey; Native chicken; Semen qualities; Cryopreservation; Post-freezing

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

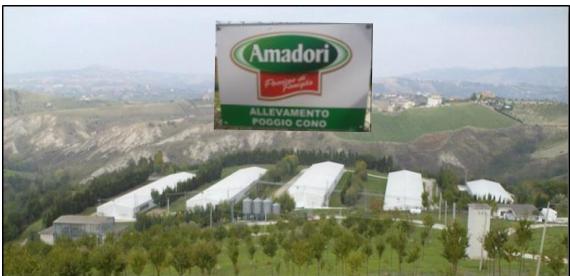


# Materials and Methods

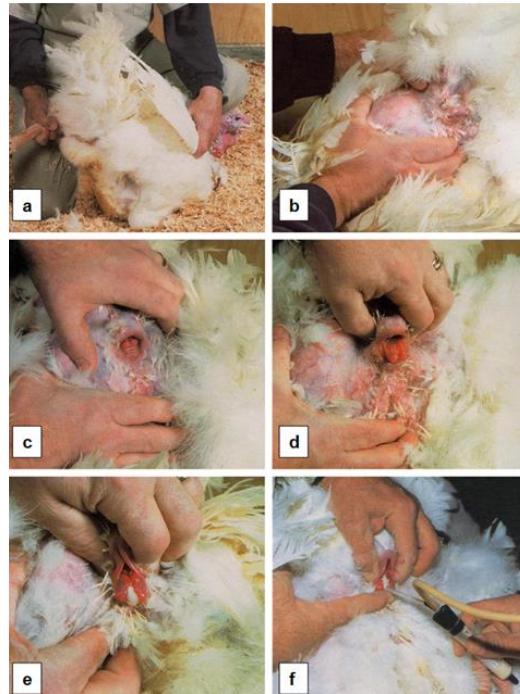
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## 1. Animals

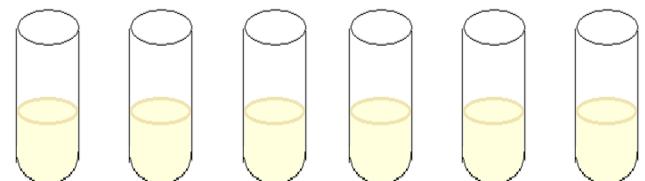
40 ♂



## 2. Semen collection

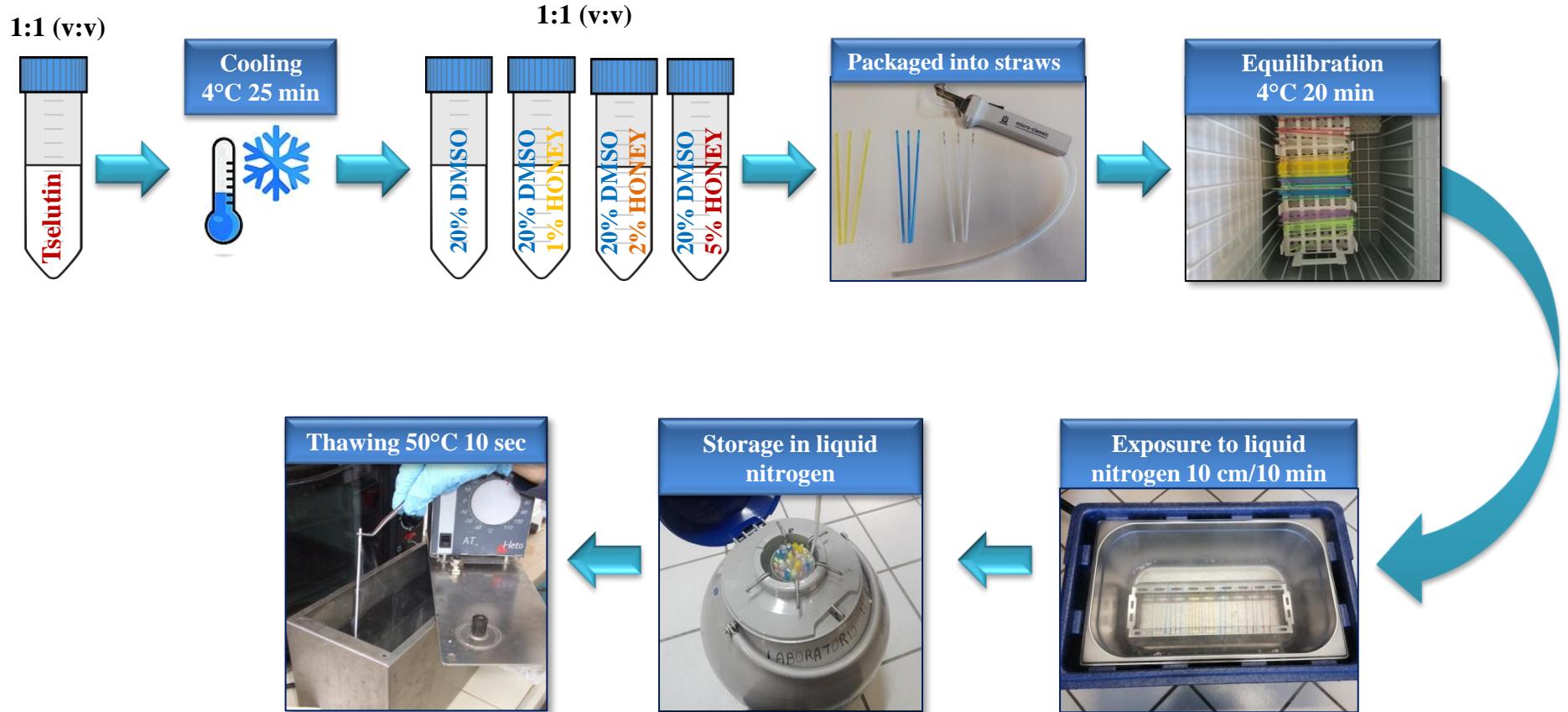


6 POOLS



# Materials and Methods

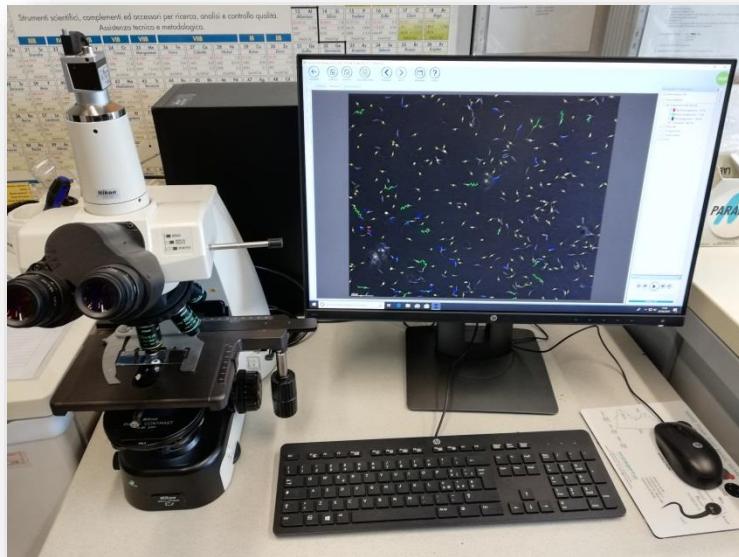
## 3. Freezing protocol



# Materials and Methods

## 4. Sperm quality - Motility

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



- 1** Dilution  
 $100 \times 10^6$  spz/mL



- 2** Incubation  
38°C 5 min



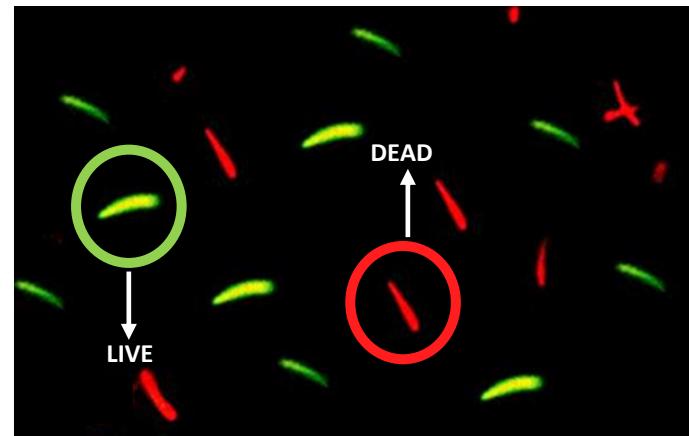
- Total Motility (%)
- Progressive Motility (%)
- VCL ( $\mu\text{m/s}$ )
- VSL ( $\mu\text{m/s}$ )
- VAP ( $\mu\text{m/s}$ )
- LIN (%)
- STR (%)

# 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<sub>2</sub>O     → 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



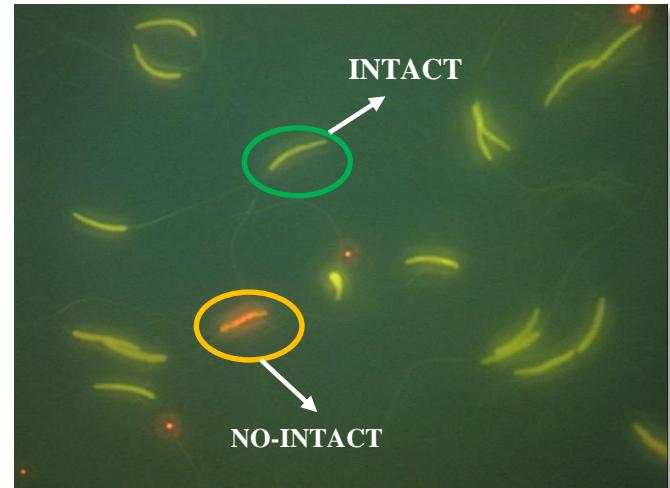
$$\frac{\% \text{ viability}}{\text{osmotic resistance}} \rightarrow \frac{\text{Green spermatozoa}}{\text{Green spermatozoa} + \text{Red spermatozoa}} \times 100$$

# 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



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

# Materials and Methods

## 5. Statistical analysis



Sperm quality parameters were analyzed by ANOVA



DUNCAN comparison test



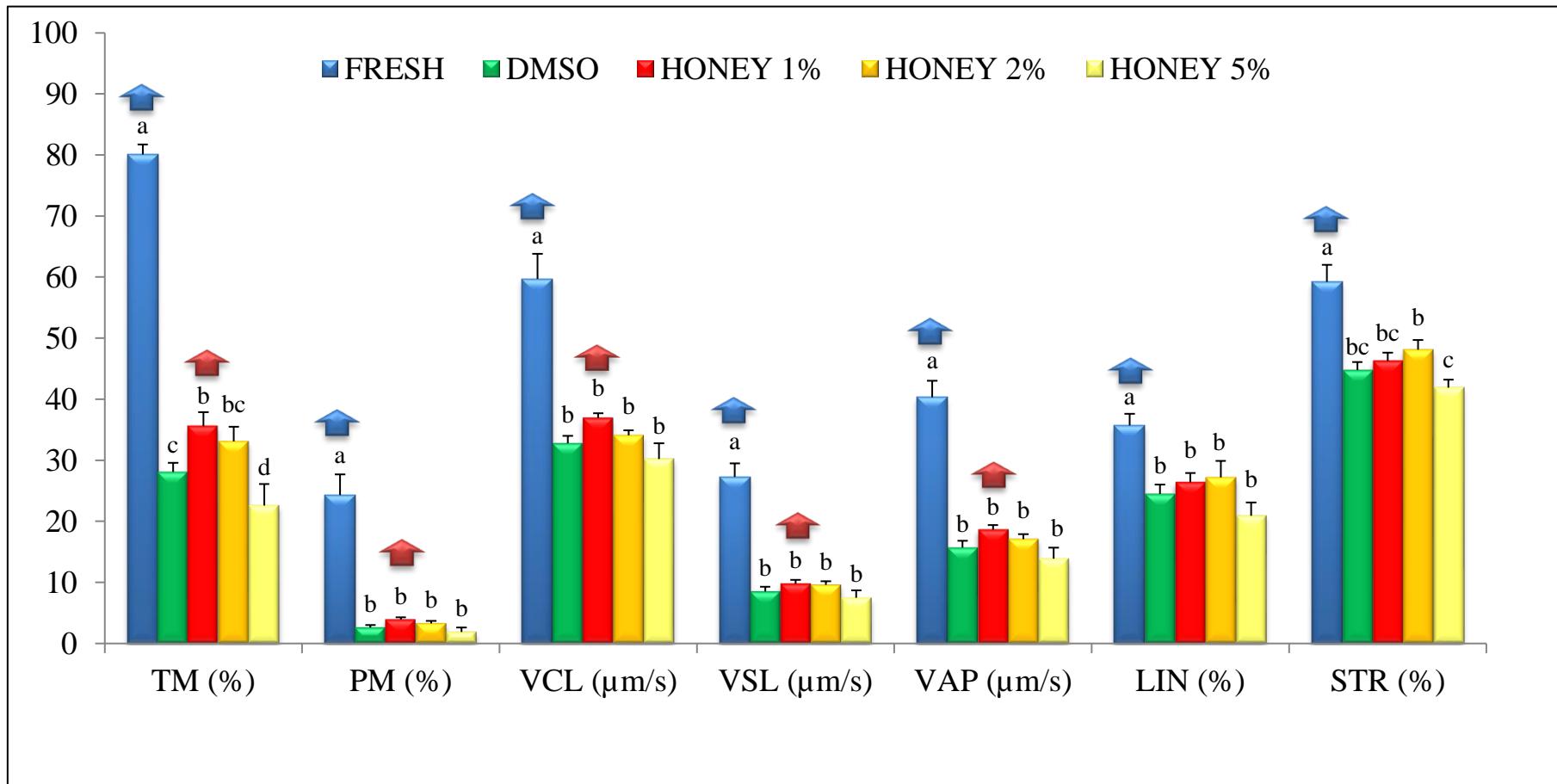
Significance was set at  $P < 0.05$

SPSS Data Editor window showing a dataset with 30 rows and 10 columns. The columns are labeled TRAT, MOTTOT, MOTPR, VCL, VAP, VSL, STR, LIN, var, and var. The data includes various numerical values for each row.

SPSS Output viewer showing the One-Way ANOVA results. The output includes:

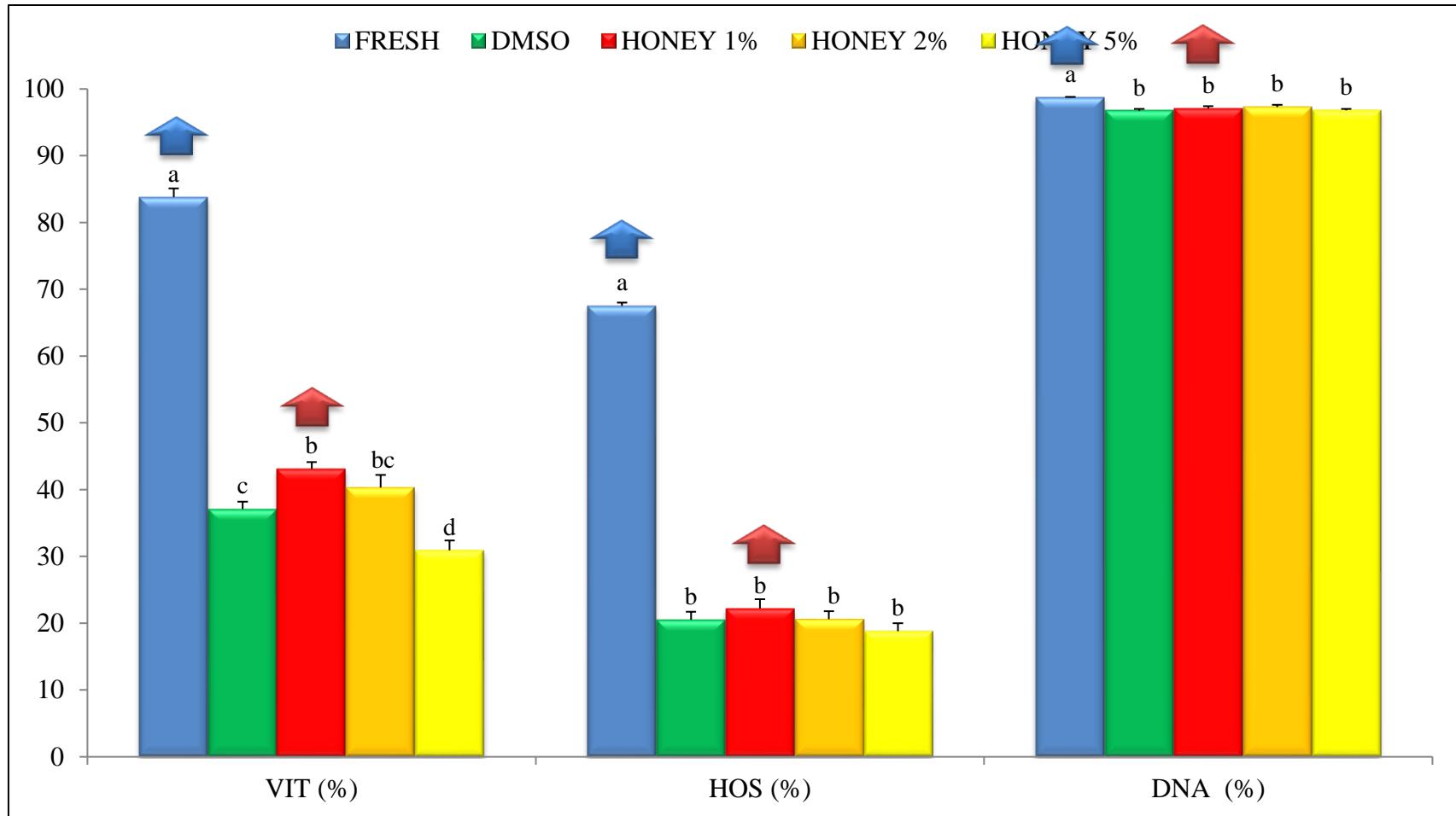
- ANOVA:** ANOVA table for MOTTOT, MOTPR, VCL, VAP, VSL, and LIN. For example, for MOTTOT, Between Groups Sum of Squares is 10978.877, df is 4, Mean Square is 2744.719, F is 88.958, and Sig. is .000.
- Post Hoc Tests:** DUNCAN comparison test results for each parameter.
- Homogeneous Subsets:** Results for creating homogeneous subsets based on the DUNCAN test.

# Results



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

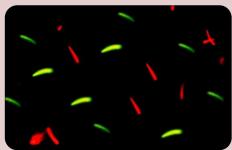
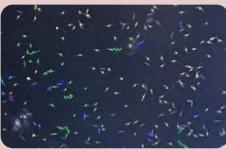
# Results



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

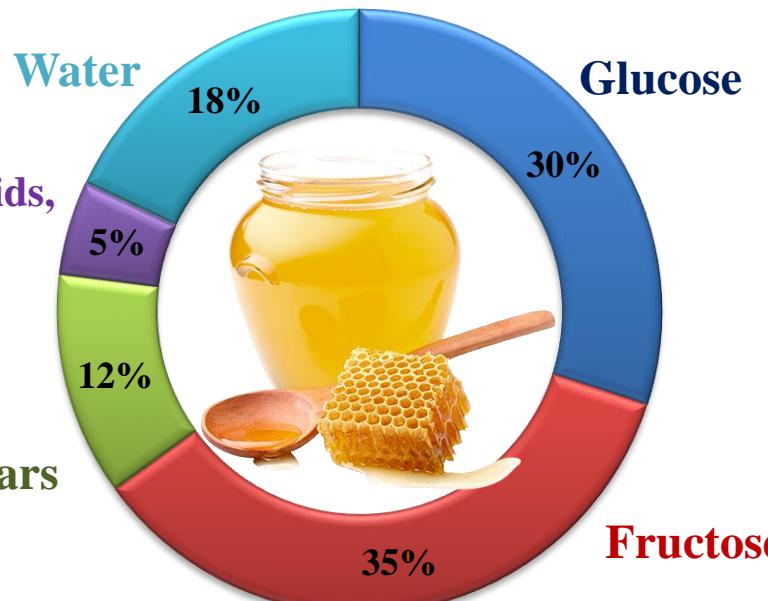
# Discussions and Conclusions

Freezing/thawing process

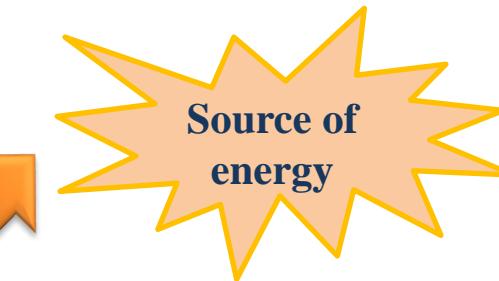
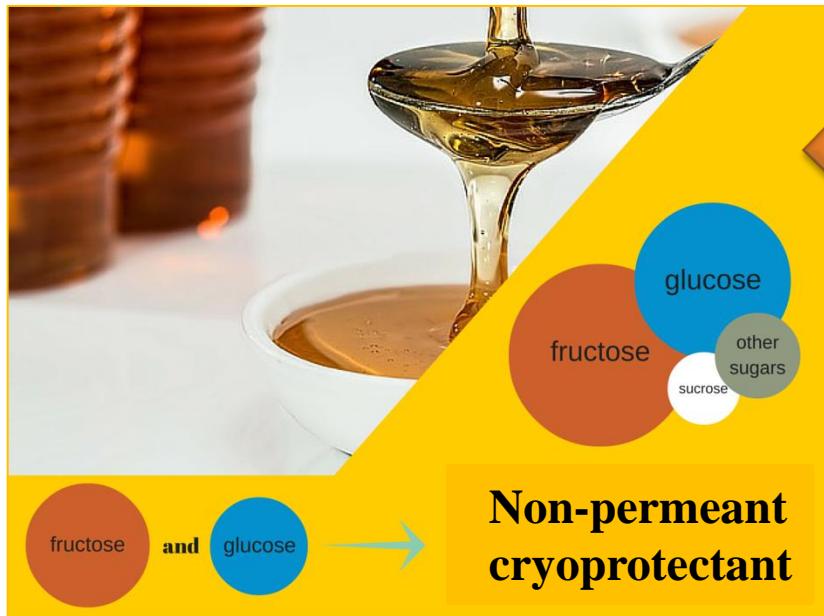


How can the honey improve the post-thaw quality of turkey spermatozoa?

Mineral, vitamins, flavonoids, phenolic compounds

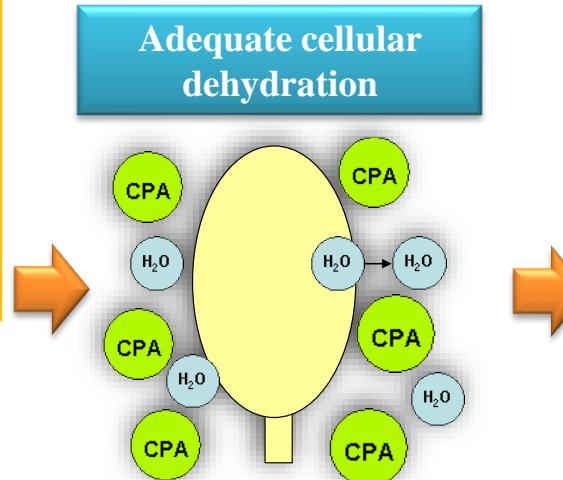


# Discussions and Conclusions

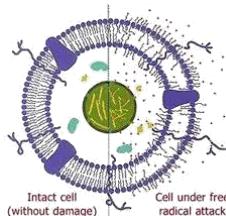
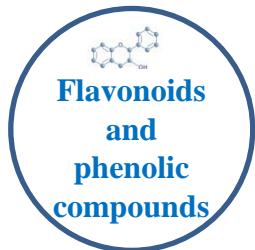


Gil et al., 2010; Rahardhianto et al., 2012

Adequate cellular dehydration



Reducing ice-crystals



El-Shehhtawy 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



**1<sup>st</sup>**  
Italian Semen  
Cryobank of  
Autochthonous Poultry

Breeds



A photograph of several chickens and turkeys in a grassy field. In the foreground, there is a brown hen with a white comb and wattle. Behind her is a large white turkey with a dark neck and a small white bird. To the right, there is a guinea fowl with a dark body and white spots. In the background, there are more turkeys and chickens of various colors, including white and brown. The sun is shining brightly, creating strong shadows and highlights on the birds' feathers.

Thank you  
for  
your attention