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Workshop n. 7 – Advances in sperm cryopreservation technology for conservation of avian genetic resources

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Relation between cryoprotectant concentration, sperm functional integrity and fertilizing ability in frozen/thawed chicken sperm

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Research background

Factors related to semen *in vitro* processing have been studied to improve the recovery of viable and motile chicken sperm after cryopreservation in order to set up a successful chicken semen cryopreservation procedure:

- inclusion of glycine into Lake's freezing extender (LFE)
- temperature gradient for freezing in liquid nitrogen vapours
- inclusion of non-permeant cryoprotectant into LFE
- thawing temperature
- type of cryoprotectant and its concentration

The cryopreservation procedure was required to implement the Italian Semen Cryobank for Autochthonous Poultry Breeds, corresponding to one of major aims in TuBAvI project ((2018-2024 www.pollitaliani.it)

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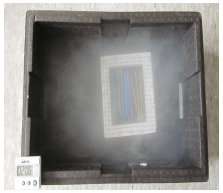
Aim and treatments

- To compare **dimethylacetamide *vs* N-methylacetamide**
- To compare decreasing cryoprotectant concentration ⇢ **6 *vs* 4 *vs* 2 *vs* 0 %**

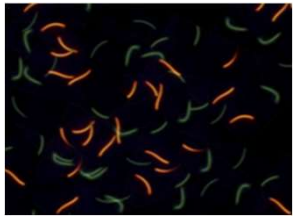
Materials and Methods

Egg-type breeders

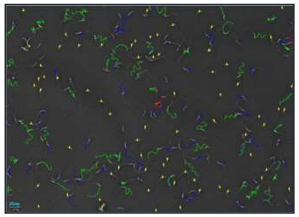


In vitro sperm parameters:

- Sperm membrane integrity
- Total motility
- Progressive motility
- Sperm kinetic parameters



SYBR14/PI test



SCA system

In vivo parameters:

- Fertility
- Embryo viability

1 insemination with $250x10^6$ sperm, eggs collected for 5 days

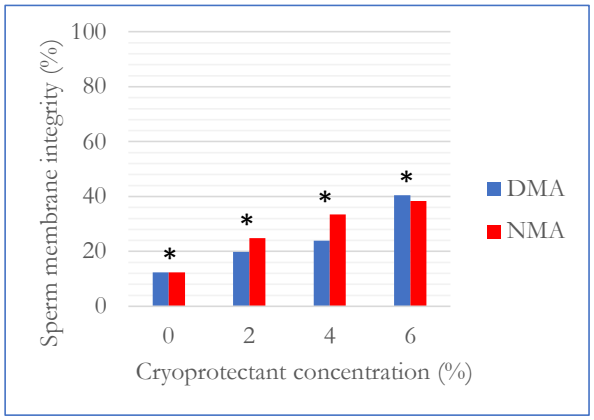
Candling

Statistics: PROC MIXED SAS for *in vitro* data, Chi-square test for *in vivo* data

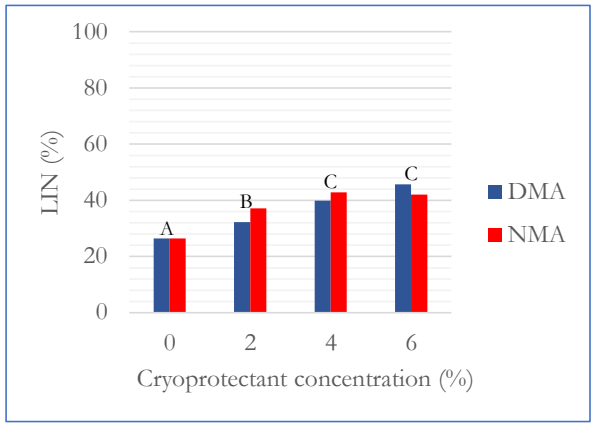
- Acrosome integrity
- Mitochondria function

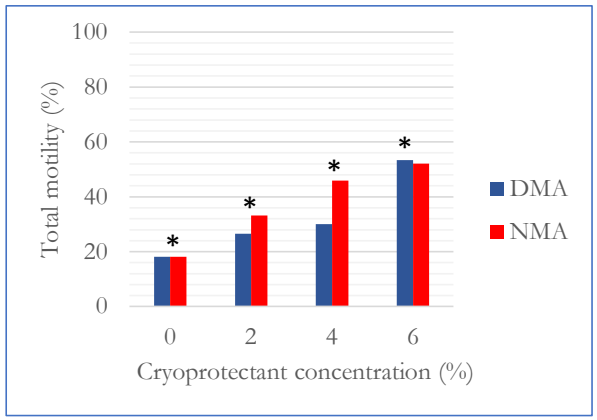
Statistics: Principal Component Analysis

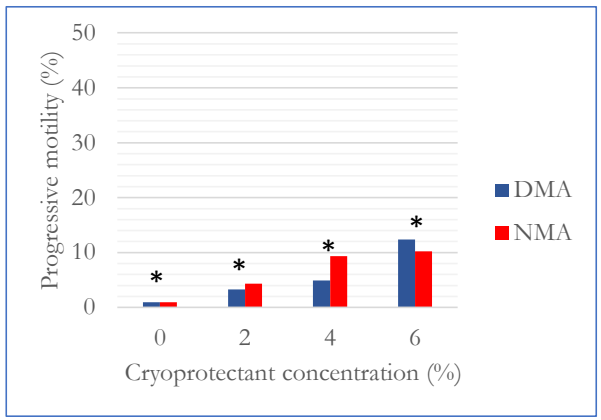
Results – Sperm quality variables

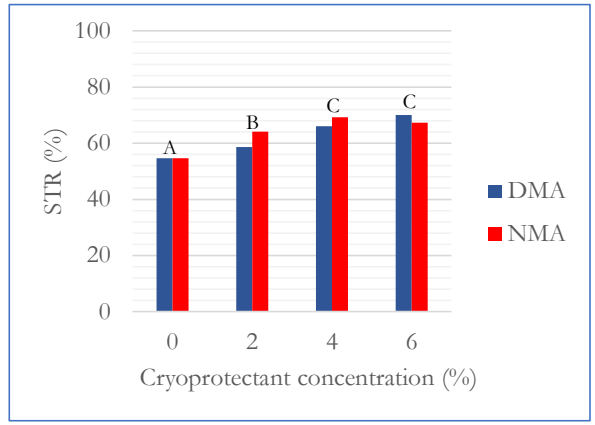


- Type of **cryoprotectant not significant**
- **Cryoprotectant concentration higly significant**

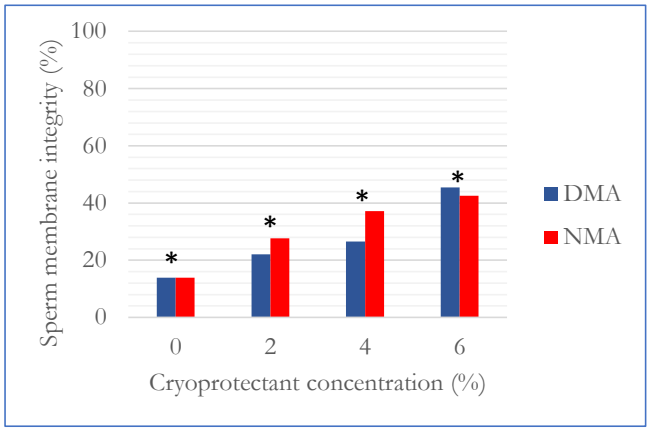


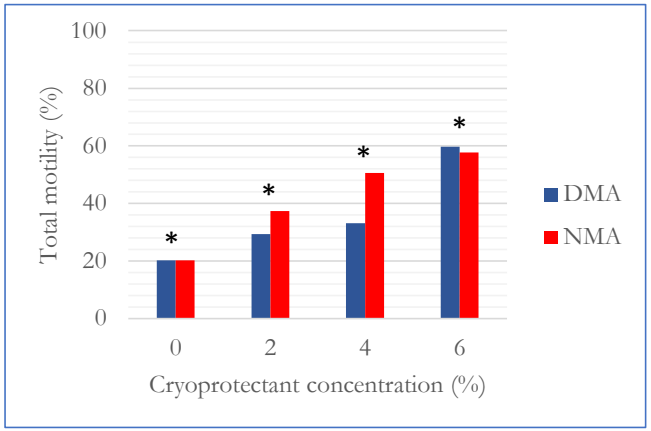






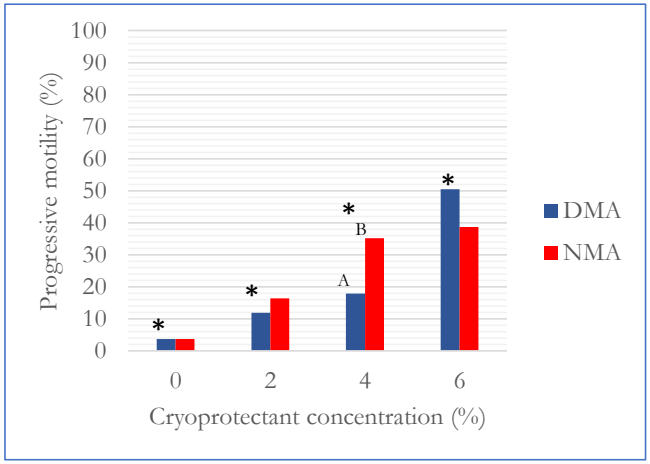
Results - Recovery rates





Cryoprotectant not significant and cryoprotectant concentration higly significant

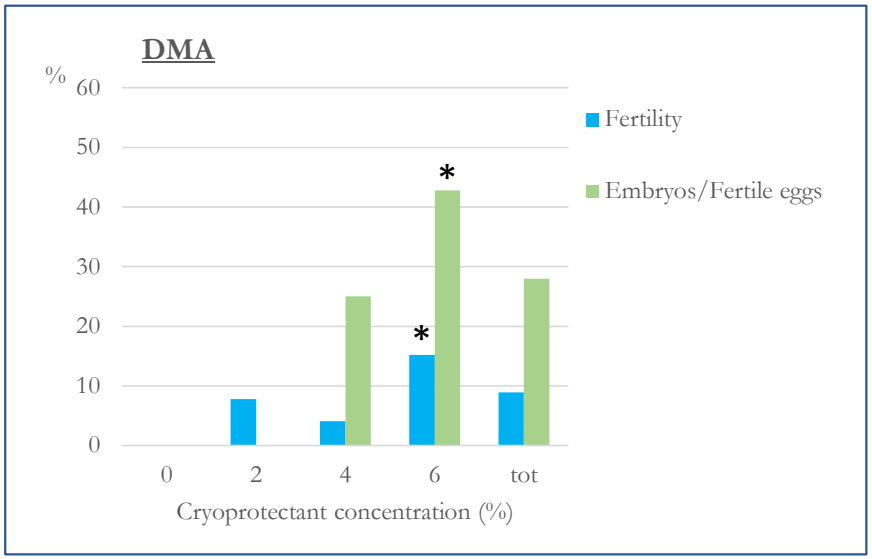
Significant interaction cryoprotectant*cryop. concentration only for progressive motility

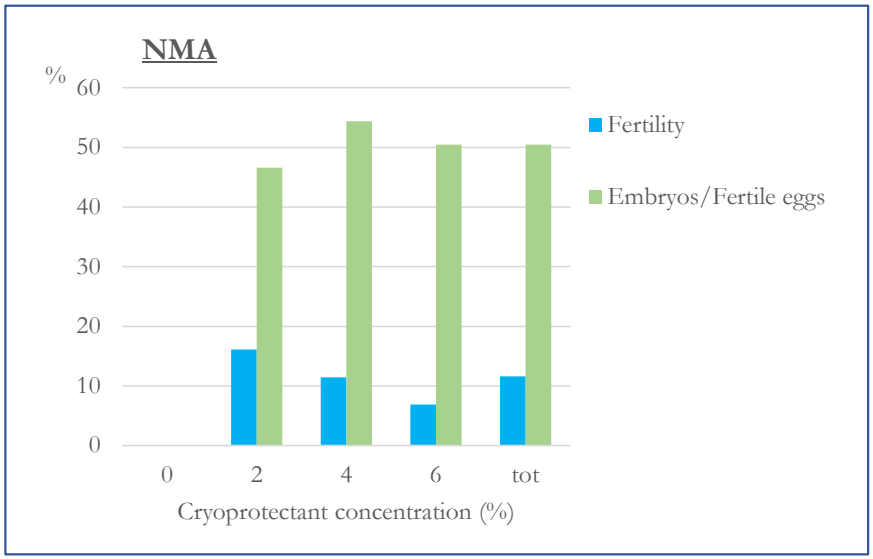


$$\text{Recovery} = \frac{\text{mean value thawed semen}}{\text{mean value fresh semen}} \times 100$$

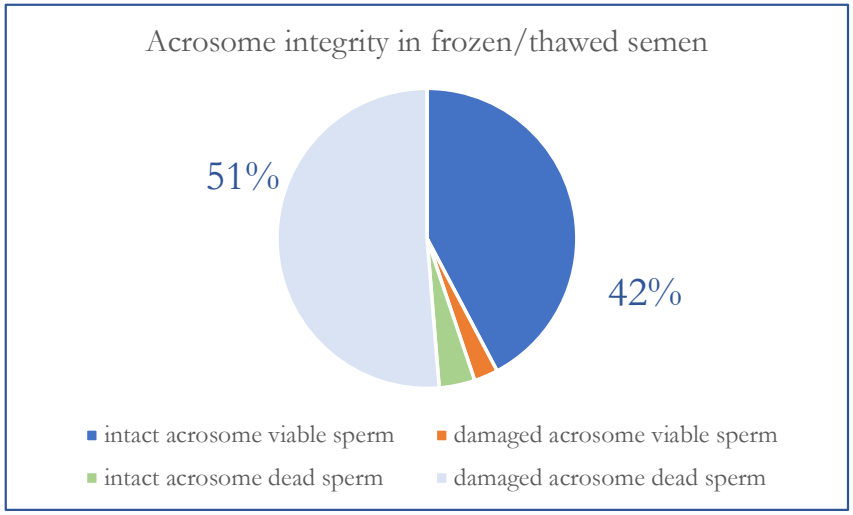
Results – Fertility and embryo viability

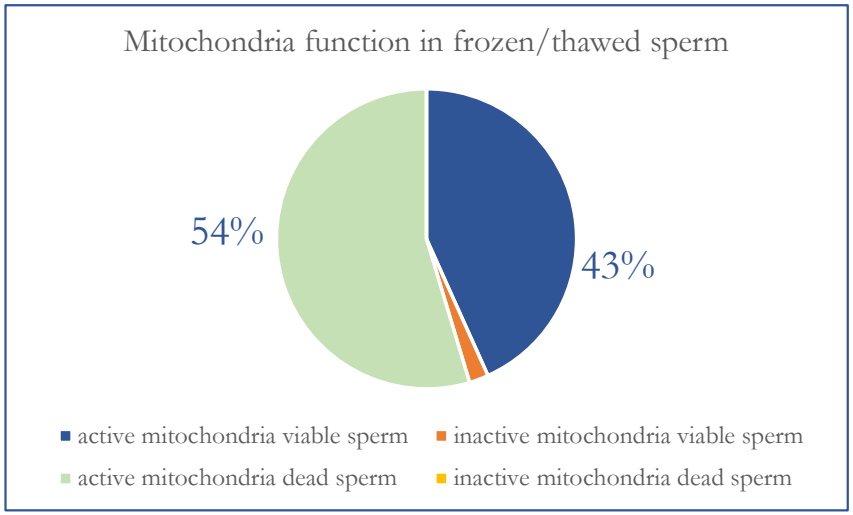
Different effect of cryoprotectant concentration between DMA and NMA





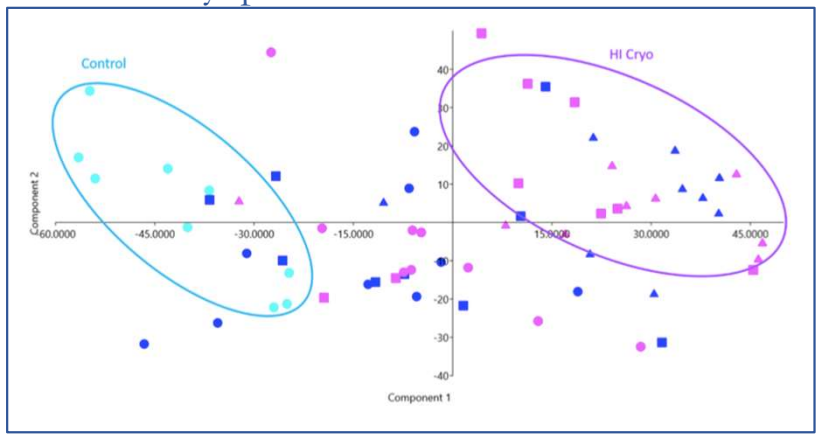
Results – Acrosome and mitochondria integrity





Results Principal Component Analysis

Scatter Plot PCA cryoprotectant and cryoprotectant concentration.



Turquoise= Control; Blue= DMA; Fuchsia=NMA
Spot=2%; square=4%; triangle=6%.

80% variance is defined by PC1 (56%) and PC2 (24%)

Variance mainly related to total motility and sperm membrane integrity

Conclusions

- 2% NMA adopted ⇢ higher *in vivo* results at the lowest cryoprotectant concentration

Further trials to :

1. deeper investigate relation between *in vitro* and *in vivo* parameters
2. improve embryo viability after A.I. of cryopreserved chicken semen

Cryopreservation procedure for chicken semen adopted to set up the Italian Semen Cryobank of Autochthonous Chicken and Turkey Breeds

- Dilution to 1.5×10^9 sperm/mL with Modified pre-Freezing Lake (MFL) diluent (Mosca et al. 2016a);
- Cooling at 4 °C for 20 min;
- Dilution at 1.0×10^9 sperm/mL with MFL diluent added with NM 2% final concentration;
- Equilibrium at 4 °C for 1 min;
- Packaging into straws (0.25 mL): 250×10^6 sperm/straw;
- Freezing by exposure of straws 3 cm above liquid nitrogen bath for 10 min;
- Transfer and storage of straws into liquid nitrogen cryotank;
- Thawing in a thermostatically controlled bath at 5 °C for 100 s.

Thank you for your attention

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