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Data Article

Flow cytometry data on the effect of dimethylacetamide and N-methylacetamide used at different concentrations on the quality of cryopreserved chicken semen

Manuela Madeddu*, Luisa Zaniboni, Stefano Paolo Marelli, Stefano Comazzi, Silvia Cerolini

Department of Veterinary Medicine and Animal Science, University of Milan, via dell'Università 6, Lodi 26900, Italy

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ABSTRACT

This article includes supporting data regarding the research article entitled "Concentration dependent effect of dimethylacetamide and N-methylacetamide on the quality and fertility of cryopreserved chicken semen" (Zaniboni et al., 2022). The effect of two permeant-cryoprotectants (CPA), dimethylacetamide (DMA) and N-methylacetamide (NMA) used at different concentrations (0, 2, 4 and 6%) on the quality of post thaw rooster semen was assessed. Ejaculates were processed according to 7 treatments: Lake pre-freezing+0.1 M trehalose (LPF-T) (control treatment), LPF-T+2% DMA (DMA2), LPF-T+4% DMA (DMA4), LPF-T+6% DMA (DMA6), LPF-T+2% NMA (NMA2), LPF-T+4% NMA (NMA4), LPF-T+6% NMA (NMA6). Sperm acrosome integrity and mitochondrial activity were investigated in frozen-thawed semen with the use of the flow cytometry technique. Only the mitochondrial activity was significantly affected by the different cryoprotectant concentrations.

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* Corresponding author.
E-mail address: manuela.madeddu@unimi.it (M. Madeddu).

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Specifications Table

Subject	Biological Sciences
Specific subject area	Cryopreservation of chicken semen
Type of data	Table and scatter plot
How the data were acquired	Data were acquired with the following flow cytometer: BriCyte E6, Mindray, Shenzhen, China
Data format	Raw Analyzed
Description of data collection	The data is readable in FCS format. Each file is the measurement of a patient sample and for each sample a control tube in which stainings (respectively Peanut Agglutinin (PNA) and Propidium Iodide or Rhodamine 123 and Propidium Iodide) were omitted at the same incubation conditions was added. A minimum of 10,000 cells (events) were collected and analysis were carried out on a dual color scattergram gated of the population of putative spermatozoa based on FS and SS light scatter. Control tubes were used to define cutoff values and the results were reported as the percentage of events falling in each of four different quadrants.
Data source location	Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi (Italy)
Data accessibility	Repository name: Mendeley Data Direct link to the dataset: https://data.mendeley.com/datasets/r24mpzj8wp/1
Related research article	L. Zaniboni, M. Madeddu, F. Mosca, A. Abdel Sayed, S.P. Marelli, M. Di Iorio, N. Iaffaldano, S. Cerolini. Concentration dependent effect of dimethylacetamide and N-methylacetamide on the quality and fertility of cryopreserved chicken semen, Cryobiology, Volume 106, June 2022, Pages 66-72. doi:https://doi.org/10.1016/j.cryobiol.2022.04.001.

Value of the Data

- Data showed in this manuscript are complementary and improve the analyzes previously carried out; they provide new information on the comparative effect of dimethylacetamide and N-methylacetamide used at different concentrations on the functional integrity of cryopreserved chicken semen.
- Data suggest the efficacy of flow cytometry technique for analysis of intracellular structures of cryopreserved sperm and then highlight the most severe cryodamages.
- Data are useful to provide clues to develop improved chicken semen cryopreservation procedure and to understand which in vitro analyzes are required to obtain meaningful results.

1. Objective

The effect of two CPAs, DMA and NMA used at different concentrations (0, 2, 4 and 6%) on the quality of post thaw poultry semen was assessed. Sperm acrosome integrity and sperm mitochondrial activity were investigated in frozen-thawed semen with the use of the flow cytometry. These data contributed to the collection of further sperm parameters useful for the qualitative assessment of thawed chicken sperm, making the analyzes already performed in the previous study more in-depth.

2. Data Description

Data contain the acrosome integrity and mitochondrial activity recorded in cryopreserved chicken semen (Table 1). The type of CPA and its interaction with CPA concentration did not significantly affect the variables analyzed, whereas CPA concentration significantly affected only live sperms with active mitochondria and dead sperms with active mitochondria. The proportion of live sperms with active mitochondria was significantly improved in presence of DMA, irrespec-

Table 1
Acrosome integrity and mitochondrial activity (LSMeans±S.E.) in frozen-thawed chicken semen cryopreserved with different concentrations of permeant cryoprotectant dimethylacetamide (DMA) and N-methylacetamide (NMA).

Treatments[1]								
Sperm variable	CTR0	DMA2	DMA4	DMA6	NMA2	NMA4	NMA6	S.E.
Live with intact acrosome (%)	35.48	36.10	45.46	44.47	45.5	36.58	40.94	1.7
Live with ruptured acrosome (%)	2.99	2.80	2.58	1.57	2.93	2.09	3.51	0.2
Dead with intact acrosome (%)	1,95	4.45	1.97	3.93	2.54	6.75	4.53	0.7
Dead with ruptured acrosome (%)	59.56	56.60	49.97	49.88	48.97	54.54	50.98	1.5
Live with active mitochondria (%)	22.74[a]	46.29[b]	39.53[b]	44.96[b]	42.90[b]	35.10[ab]	48.88[b]	3.4
Live with inactive mitochondria (%)	1.49	2.39	2.06	4.95	1.62	1.53	4.13	0.5
Dead with active mitochondria (%)	75.73[a]	51.32[b]	58.34[b]	50.06[b]	55.44[b]	63.36[ab]	46.96[b]	3.7
Dead with inactive mitochondria (%)	0.05	0.03	0.03	0.01	0.02	0.02	0.03	0.005

[a,b,c] Values within a row with different superscripts shows a significant difference P<0.01 between DMA and CRT and a significant difference $P < 0.05$ between NAM and CTR.
[1] CTR0: 0% cryoprotectant; DMA2: 2% DMA; DMA4: 4% DMA; DMA6: 6% DMA; NMA2: 2% NMA; NMA4: 4% NMA; NMA6: 6% NMA.

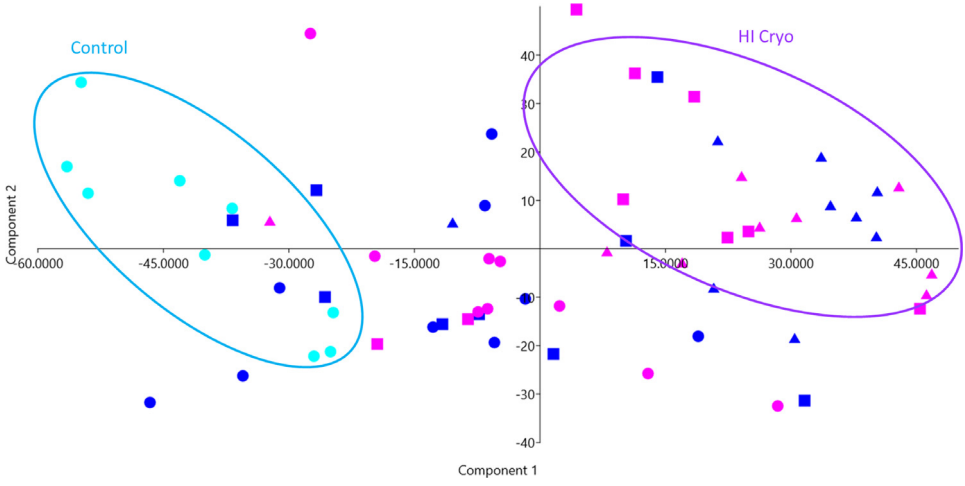

Fig. 1. Scatter Plot of principal component analysis (PCA) with different concentrations of permeant cryoprotectant dimethylacetamide (DMA) and N-methylacetamide (NMA).

tive of its concentration; in contrast in NMA treatments mitochondrial activity was significantly improved in NMA2 and NMA6 compared to control group.

The new dataset was merged with the previous article dataset and the Principal Component Analysis (PCA) was carried out in order to identify the more influencing variables and a scatterplot was produced (Fig. 1). The PCA gives objective information on the effects of the two cryoprotectants and their concentrations on the analysed samples. The variance is defined by PC1 and PC2, the first two Principal Components report 79.61% (PC1=55.53%; PC2=24.08%) of the variance; PC1 is mainly influenced by total sperm motility (0.55) and sperm membrane integrity (0.40). On PC2 the variance is linked to dead sperms with active mitochondria and live sperms with active mitochondria (0.50; -0.45%) parameters. The scatter plot reveals cryoprotectants effects on frozen/thawed semen samples. In particular, control group samples cluster on PC1 and PC2, quadrants 3 and 4 (Fig. 1). Some DMA and NMA samples with different cryoprotectant concentrations share the same area. The clustering ability of DMA and NMA samples at higher concentrations is clear on PC1 and two almost exclusively grouping in quadrant 1 and 2. PCA results underline the effective cryoprotective action of DMA and NMA at higher concentrations on integrity of cellular and intracellular sperm membranes. In addition,

the presence of the overlapping areas suggests a sample specific reaction to the cryopreservation process.

Every sign represents a sample, every colour a cryoprotectant (Turquoise= Control; Blue= DMA; Fuchsia=NMA) every shape a concentration (spot=2; square=4; triangle=6). Cryoprotectant specific lines circling the samples of each cryoprotectant groups have been drawn to better visualize the clusters.

3. Experimental Design, Materials and Methods

3.1. Semen Samples

Semen samples prepared and stored during the experimental study already reported in the published manuscript [1] were used. In particular the straws used for analysis were cryopreserved with different concentrations of CPAs in order to provide the following treatments: (1) LPF-T (Lake pre-freezing extender including 0.1 M trehalose) with no CPA (control treatment, CTR-0); (2) LPF-T added with 2% (v:v) DMA final concentrations (DMA-2); (3) LPF-T added with 2% (v: v) NMA final concentrations (NMA-2); (4) LPF-T added with 4% (v:v) DMA final concentrations (DMA-4); (5) LPF-T added with 4% (v:v) NMA final concentrations (NMA-4); (6) LPF-T added with 6% (v:v) DMA final concentrations (DMA-6); (7) LPF-T added with 6% (v:v) NMA final concentrations (NMA-6). A total of 7 different straw colours were used according to the 7 different treatments. Seven straws for treatments were thawed at 5 ◦C for 100 s [2].

3.2. Semen Evaluation

The characteristics of the sperm were evaluated in the frozen-thawed samples.

The measurements were done on a BriCyte E6 (Mindray, Shenzhen, China) flow cytometer. The fluorescent probes used in the experiment were excited by an Argon ion 488 nm laser. Acquisitions were done using the MRFLOW software. The non-sperm events were gated out based on scatter properties and not analyzed. A total of 10,000 events were analyzed for each sample.

3.3. Acrosome Integrity

Acrosomal damage was assessed as described by Partyka et al. [3] with minor modifications. In brief, semen was diluted with NaCl 0.9% to a concentration of 50×10^6 spz/mL. Portions (500 µL) of the diluted semen were placed into cytometric tubes and 1 µL of Phycoerythrin labelled with Peanut agglutinin solution (PE-PNA) (Invitrogen) (10 µg/mL) was added. Samples were mixed and incubated for 5 min at room temperature in the dark. Cells were counterstained with 1 µL of Propidium Iodide commercial solution 2 min before analysis. Acrosome disruption were identified for the positivity to PE-PNA (yellow fluorescence) and dead cells for the positivity to Propidium Iodide (red fluorescence). Four populations of spermatozoa were showed: live cells with intact acrosome, live cells with ruptured acrosome, dead cells with intact acrosome and dead cells with ruptured acrosome .

3.4. Mitochondrial Function

The percentage of spermatozoa with functional mitochondria was estimated as described by Partyka et al. [3] with minor modifications. In brief, semen was diluted with NaCl 0.9% to a concentration of 100×10^6 spz/mL. Portions (1000 µL) of the diluted semen were placed into cytometric tubes and 20 µL of Rhodamine 123 (R123, Invitrogen) working solution (0.01 g/mL)

obtained by dilution Rhodamine 123 stock solution (0.1 g/mL; 0.1 g Rhodamine 123 was diluted in 1 mL NaCl 0.9%) in NaCl 0.9% at the ratio of 1:10. Samples were mixed and incubated for 20 min at room temperature in the dark. Cells were counterstained with 1 µL of Propidium Iodide commercial solution 2 min before analysis. Functional mitochondria were identified for the positivity to Rhodamine 123 (green fluorescence) and dead cells for the positivity to Propidium Iodide (red fluorescence). Four populations of spermatozoa were identified: live spermatozoa with active mitochondria, live spermatozoa with inactive mitochondria, dead spermatozoa with active mitochondria and dead spermatozoa with inactive mitochondria.

3.5. Statistical Analysis

Analysis of variance of sperm variables (acrosome integrity and mitochondrial function) recorded in frozen/thawed semen samples was performed using the GLM procedure of SAS [4]. The CPA (DMA vs NMA), its concentration (0, 2, 4 and 6%) and the relative interaction were the sources of variation included in the model. The Student's *t*-test was used to compare LSMeans.

PCA was conducted on different treatments with sperm parameters as variables. A scatterplot was produced.

Ethics Statements

The sperm donor roosters were handled in accordance with the principles presented in Guidelines for the Care and Use of Agricultural Animals in Research and Teaching [5]. The Animal Welfare Committee of the University of Milan evaluated and approved the experimental protocol and bird management (OPBA_134_2017).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Datainbrief_dataset (Original data) (Mendeley Data).

CRediT Author Statement

Manuela Madeddu: Formal analysis, Data curation, Writing – original draft; **Luisa Zaniboni:** Conceptualization, Methodology, Data curation, Writing – review & editing; **Stefano Paolo Marelli:** Formal analysis, Writing – review & editing; **Stefano Comazzi:** Software, Data curation, Writing – review & editing; **Silvia Cerolini:** Conceptualization, Supervision, Writing – review & editing.

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cryobiol.2022.04.001.

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