

## Freezing poultry semen: effects of CPA concentration × cooling rate, and other factors

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Cryopreservation of chicken semen is used in gene banks for ex situ conservation of genetic diversity, or in the breeding industry to conserve selection lines. However, optimization of freezing medium and freezing protocol is necessary as cryo-resistance may be low in specific breeds. In the present study a number of CPAs were compared, and effects of DMA concentration, cooling rate, and other variables were studied. The effect of osmolality of the base extender (no CPA) on sperm cells longevity during 5 °C storage was first tested using extenders with equal composition in terms of solute ratios, but having osmolalities ranging from 290-410 mOsm/kg. Higher osmolalities had a strong negative effect on sperm motility, which was only partly reversible, indicating permanent injury. Six related CPAs (methylformamide, methylacetamide, dimethylformamide (DMF), dimethylacetamide (DMA), propane-1,2-diol, and diethylformamide) were first pre-screened at 0.6 M for freezing semen from individual cocks (n=10) in 0.25-ml straws at a cooling rate of 250 °C/min. Post-thaw % motile and % live sperm were highest with DMA and DMF. Finally, semen from individual cocks or pooled semen was frozen in 0.25-ml straws, using cooling rates (CRs) of 4, 50, 250, and 440 °C/min and [DMA] of 0.4, 0.6, 1.0, and 1.5 M. Results of microscopical sperm assessment were presented elsewhere. Data from flow cytometry, Tunel analysis, and CASA motility analysis will now be presented. Results show clear effects of both CR and [DMA]. Percentage motile and % live sperm were highest for CRs 50-250 °C/min. Higher DMA concentrations gave better post-thaw sperm survival. However, longevity of the sperm at 1.0 and 1.5 M DMA was compromised. Therefore, [DMA] may best be 0.6-1.0 M at a CR of 50-250 °C/min. This work was part of the IMAGE project which received funding from the European Union's Horizon 2020 Research and Innovation Programme under the grant agreement no. 677353.