

## **FREEZING POULTRY SEMEN; EFFECTS OF CPA CONCENTRATION X COOLING RATE; 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, post-thaw fertility may be low, especially in endangered breeds. Glycerol is a good cryoprotective agent (CPA), but must be removed before insemination as it acts as contraceptive in the hen. Adequate fertility was obtained earlier with dimethylacetamide (DMA) as CPA. In the present study, a number of CPAs were compared, and DMA concentration, cooling rate, and other variables were studied.

The effect of osmolality of the base extender (no CPA) on sperm cells was first tested during pre-freeze 5°C storage 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 of the cells. Six related CPAs (methylformamide, methylacetamide, dimethylformamide (DMF), DMA, propane-1,2-diol, and diethylformamide) were first pre-screened at 0.6M 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, in more detailed factorial experiments, 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 1.5M. There were clear effects of both CR and [DMA], but no evidence for interaction of CR x [DMA]. Percentage motile and % live sperm were highest for CRs 50-250°C/min. Higher DMA concentrations gave better post-thaw sperm survival, with relative % motile sperm 77±6% (mean±SE; four replicates) at 1.0M DMA and CR 250. However, longevity of the sperm at 1.0 and 1.5M DMA was compromised. Therefore, [DMA] may best be 0.6-1.0M at a CR of 50-250°C/min.

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