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# Changes in the quality of chilled and frozen-thawed chicken sperm when heated near the temperature of the female reproductive tract

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Fertilisation capacity of cryopreserved chicken sperm varies widely in the literature and many questions are unresolved about sperm changes in the female reproductive tract. Considering that hen's temperature is around 41 °C, the present study investigated the effect of warming chilled and frozen-thawed sperm from 5 °C to 37 °C or to room temperature. Experiment 1, pooled semen (5 roosters/pool; n=4), extended with ASG diluent with 0.6 M DMA was aliquoted (0.22 mL) in glass tubes and placed at 5°C for 1h. One tube/pool was fixed with formaldehyde while another was immersed in 37 °C water (1 min) and fixed. Experiment 2, pooled semen (5 roosters/pool; n=9) was cryopreserved (0.6 M DMA) in 0.25 straws at 50 °C/min. Two straws per pool were thawed in 5 °C water and semen was collected in different glass tubes. One tube was fixed at 5 °C and, the other, after immersion in 37 °C water (1 min). Acrosome integrity (Acr%) was assessed with methyl blue staining. Experiment 3, 20 hens (n=10/group) were inseminated (≈100 million sperm); group 1: 4 times (twice weekly) with extended semen chilled at 5°C for 1h (CES) + 3 times (in one week) with room temperature extended semen (RTES); group 2: 7 times with neat semen (NS). Acr% of chilled semen was not affected by warming (exp. 1), but Acr% of frozen-thawed semen (exp. 2) decreased from 80.9 ±2.3% (5°C) to 11.1 ± 2.18% (37°C). Fertility in inseminations 1-4 was lower with CES (3.7%) than with NS (48.2%) but increased to 54% with RTES (inseminations 5-7) while NS reached 71.4%. In conclusion, sperm damage caused by chilling/freezing-thawing may only be detected after subsequent rewarming. Mere chilling (and rewarming in the hen) caused a drastic decrease in fertility.

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