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Artificial insemination of cryopreserved chicken semen: do different concentration doses affect fertility?



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### Aim

To study the relation between fertility and the insemination concentration dose (ID) of cryopreserved chicken semen.

# Materials and Methods

Thirty-six adult commercial line cockerels were housed in single cages and kept according to the standard management guidelines for chicken breeders. After a training period, ejaculates were routinely collected twice weekly. On each day of collection, all the ejaculates were pooled into one semen sample and processed for cryopreservation according the following steps: 1) dilution in pre-freezing modified Lake diluent supplemented with trehalose 0.1 M (LD); 2) equilibration at 4° C for 30 min; 3) further dilution in LD added with N-methylacetamide, 6% final concentration, and equilibration for 1 min; 4) loading into 0.25 ml French straws; 5) freezing on a rack floating over a nitrogen bath at 3 cm of height for 10 min; 6) transfer into cryotank for storage. Before semen processing, semen samples were splitted into 3 aliquots, each one diluted to the following final sperm number (× 10%) per straw corresponding to the IDs: A) 250; B) 500; C) 750. Semen thawing was performed in ice-water bath at 5° C for 100 sec. Sperm quality (motility and kinetic parameters) was assessed in fresh and frozen/thawed semen samples. Artificial insemination was performed using semen from one straw per hen. Laying hens (n=27) were divided into 3 groups receiving different IDs according to group A, B and C. Eggs were collected from the second day after AI for 10 days, set every 3/4 days and fertility recorded at candling after 7 days of incubation. All clear eggs were open and true fertility recorded.

 Table 1 – Sperm motility and kinetic parameters measured in fresh

 and frozen/thawed chicken semen.

Quality	Fresh semen	Frozen/Thawed
parameters		semen
TM (%)	67.5	29.2
PM (%)	23.2	3.1
VCL (µm/s)	62.5	39.1
VSL (µm/s)	32.8	13.4
VAP (µm/s)	43.4	21.7
LIN (%)	52.4	33.8
STR (%)	75.5	60.4
WOB (%)	69.4	55.0
ALH (µm)	3.5	2.7
BCF (Hz)	8.6	4.4
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## **Results and Discussion**

Semen quality recorded in fresh and frozen/thawed semen samples is reported in Table 1. Sperm motility and kinetic parameters were consistent with previous data (Mosca et al., 2019) and very similar among treatments.

The overall fertility value recorded in group A, B and C was 6, 8 and 11% respectively. Fertility recorded in the following days after artificial insemination is reported in Figure 1. A different fertile period was recorded according to the IDs. In hens receiving  $250 \times 10^6$  sperm, fertile eggs were recorded only from day 2 to 4 and the highest fertility rate, corresponding to 25%, was recorded on day 3. In contrast, in hens receiving  $500 \times 10^6$  and  $750 \times 10^6$ sperm, fertile eggs were recorded from day 2 to 10 and the highest fertility rate was 17 and 20% in group B and C respectively.

**Figure 1** – Fertility recorded daily after artificial insemination of different cryopreserved semen doses.



\* TM = Total Motility, PM = Progressive Motility

## Conclusions

The ID of  $250 \times 10^6$  sperm was suitable to record *in vivo* fertility of cryopreserved chicken semen, even if the fertile period was limited to few days after artificial insemination. Increasing the ID to 500 and 750  $\times 10^6$  sperm did not improve the fertility rate of cryopreserved chicken semen, but a longer fertile period was recorded.

# References

Mosca F, Zaniboni L, Abdel Sayed A., Madeddu M., Iaffaldano N., Cerolini S. (2019) Effect of dimethylacetamide and N-methylacetamide on the quality and fertility of frozen/thawed chicken semen. Poultry Science, 0: 1-7. https://doi.org/10.3382/ ps/pez303.

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