Chicken sperm cryopreservation by the pellet method: study on sperm working concentration

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ABSTRACT

The purpose of this study was to determine the effects of sperm working concentration during cryopreservation pellet procedure for chicken semen. Semen samples were collected three times a week, by dorso-abdominal massage, from 18 Mericanel della Brianza (local Italian breed) chicken breeders. Two sperm working concentration, 1 or 1.5*10⁹ cells/ml, were studied during semen processing for cryopreservation. Each day of collection ejaculates were pooled in two semen samples, diluted in prefreezing diluent (1 or 1.5*10⁹ cells/ml) and equilibrated at 5°C for 20 minutes; then, dimethylacetamide (DMA; 6% final concentration) was added and equilibrated at 5°C for 1 minute. Following equilibration, the samples were frozen by direct dropping into a liquid nitrogen bath. Frozen semen pellets were transferred into cryovials, stored into liquid nitrogen tank, and thawed in water bath at 60°C. Sperm quality was assessed in fresh semen soon after collection (time 0) and in frozen/thawed semen pellets (time FT). The following sperm quality parameters were measured: subjective motility (%), damaged sperm (%), modified Ethidium bromide procedure using hypotonic solution – “stress test”) and viable sperm (eosin-nigrosin staining). The experimental protocol was repeated in different days of semen collection to increase the number of replicates per treatment (n=4). The data were analyzed by GLM procedure of SAS. Recovery rate of motility (RM), viability (RV) and undamaged (RU) sperm (%) after thawing was calculated. Sperm working concentration did not significantly affect sperm quality after thawing (time FT) and very similar recovery rates in sperm quality were measured processing semen at 1 and 1.5*10⁹ cells/ml (RM=36.30%, RV=39.47%, RU=30.68% and RM=32.33%, RV=35.42%, RU=36.91% respectively). Different works indicated a systematic lack of standardization in cryopreservation procedure. In order to standardize semen processing the initial semen dilution to a fixed working concentration was considered instead of the usual proportional semen dilution. In addition, processing a sperm suspension containing a known cell concentration is considered advantageous if cryopreserved semen will be used for artificial insemination (AI). We suggest to use 1.5*10⁹ cells/ml as sperm working concentration because it allows to store more cells in one semen pellet, therefore thawing step and preparation of the insemination dose for AI will be simplified. Finally, the recovery rate of quality parameters has been used to measure the efficiency of the cryopreservation procedure and it is suggested as reference parameter to compare different studies.

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The purpose of the experiment was to study the effect of sperm working concentration on sperm quality after semen cryopreservation by the pellet procedure in the chicken.

RESULTS
Sperm working concentration did not significantly affect sperm quality after thawing (Time FT) and very similar recovery rates for motility, viability and undamaged sperm (%) were found. Mean values recorded before and after cryopreservation, and the relative recovery rate are shown in Table 2.

DISCUSSION
Different works indicated a systematic lack of standardization in cryopreservation procedure. In order to standardize semen processing the initial semen dilution to a fixed working concentration was considered instead of the usual proportional dilution. In addition, processing a sperm suspension containing a known cell concentration is considered advantageous if cryopreserved semen will be used for artificial insemination (AI).

We suggest to use $1 \times 10^9$ cells/mL as sperm working concentration because it allows to store more cells in one semen pellet, therefore thawing and preparation of the insemination dose for AI will be simplified.

Finally, the recovery rate of quality parameters has been used to measure the efficiency of the cryopreservation procedure and it is suggested as reference parameter to compare different studies.