



A115E Physiology of reproduction in the male and semen technology

## **L-carnitine supplementation to UHT skimmed milk-based extender improves motility and membranes integrity of chilled ram sperm up to 96 h**

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The addition of new additives as L-carnitine (LC) to extenders could mitigate reactive oxygen species (ROS) production and improved motility and viability in chilled ram sperm as has been demonstrated in other species (e.g. human, bull, and mice). The aim of this work was to evaluate the antioxidant effect of LC on motility variables and integrity of plasma, acrosomal, and mitochondrial membranes of chilled (5 °C) ram sperm up to 96 h. Twelve pools from 36 semen ejaculates were collected by artificial vagina from 12 Merino rams (3-9 years) in four sessions during non-reproductive season (June to August). Each pool was divided into 6 aliquots and then diluted at  $200 \times 10^6$  sperm/ml in UHT-based extender (skimmed milk-6% egg yolk) supplemented either 1mM (LC1), 2.5mM (LC2.5), 5mM (LC5), 7.5mM (LC7.5), and 10mM (LC10) of LC. A control group without LC was included in each pool. Sperm motility variables were assessed by CASA system (SCA®) and total sperm with intact plasma membrane / intact acrosome / intact mitochondrial membrane (IPIAIM,%) was assessed by triple fluorescence association test (PI/PNA-FITC/Mitotracker green) at 0, 48, and 96 h. The effects of LC concentration and cold-storage time were analyzed by one-way ANOVA and Bonferroni's test ( $p < 0.05$ ). Overall, the results showed that kinetic variables and integrity of sperm membranes decreased ( $p < 0.05$ ) as cold-storage time increased in all groups. The results revealed a higher ( $p < 0.01$ ) sperm motility (SM,%) in all LC groups than control group at 48 h. However, at 96 h, both LC5 and LC10 groups showed a SM higher ( $p < 0.001$ ) than both LC7.5 and control group ( $87.9 \pm 2.2$  and  $88.0 \pm 1.8$  vs  $82.9 \pm 2.1$  and  $82.5 \pm 3.1$ , respectively). Progressive sperm motility (PSM,%) was higher with LC5 group than control group at 48 h ( $42.2 \pm 2.9$  vs  $36.7 \pm 1.8$ ) and 96 h ( $35.7 \pm 3.4$  vs  $29.0 \pm 1.7$ ). Surprisingly, straight line velocity (VSL,  $\mu\text{m/s}$ ) was improved with all LC groups compared with control group at 0h ( $p < 0.01$ ), 48h ( $p < 0.001$ ) and 96 h ( $p < 0.001$ ). Moreover, at 96h VSL ( $\mu\text{m/s}$ ) value was higher with LC7.5 group than all LC groups ( $p < 0.5$ ) and control ( $p < 0.001$ ) (LC7.5:  $87.2 \pm 4.9$  vs LC1:  $75.1 \pm 4.5$ , LC2.5:  $78.6 \pm 5.9$ , LC5:  $79.4 \pm 5.0$ , LC10:  $79.3 \pm 5.2$ , and control:  $65.4 \pm 3.4$ ). Likewise, IPIAIM percentage was higher ( $p < 0.001$ ) in all LC groups than control group during at 48 h and 96 h (LC1:  $62.3 \pm 2.0$ , LC2.5:  $66.3 \pm 1.7$ , LC5:  $63.3 \pm 2.8$ , LC7.5:  $66.5 \pm 2.4$ , and LC10:  $66.3 \pm 1.9$  vs control group:  $49.2 \pm 2.9$ ). These results revealed a kinetic-enhancer effect of LC supplementation to UHT skimmed milk-based extender, which might improve fertility following cervical insemination of sheep.

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