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### **Effect of different cryoprotectants on refrigerated sperm before cryopreservation of small ruminant semen**

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In an attempt to study different strategies on small ruminant sperm cryopreservation, first we tested diluents free from additives of animal origin as egg yolk after sperm refrigeration. In addition, as glycerol is potentially cytotoxic, we also tested the efficiency of trehalose as an alternative on the subsequent sperm cryopreservation. Therefore, this experiment was designed to assess the effect of the inclusion of 0.6 mM of butylated hydroxytoluene (BHT) or 1% (w/v) soybean lecithin or 15% (vol/vol) powered egg yolk supplemented with 5% glycerol or 100 mM of trehalose in a Tris-based medium on refrigerated sperm. Briefly, fresh ejaculates from 6 young bucks (1 year old) were collected by an artificial vagina and immediately mixed in equal quantities. Spermatozoa were washed by centrifugation, and then the pellet was split into six equal aliquots, resuspended in one of the six different extenders and refrigerated for 4 hours at 5°C before freezing. Likewise, fresh ejaculates from 8 young rams (1 year old) were collected and processed as buck semen samples. Sperm cryosurvival after refrigeration was determined by eosine-nigrosine stain and sperm motion parameters analysed by a computer-assisted sperm analysis system (ISAS®). The highest sperm viability percentage (mean  $\pm$  SE, n = 6) on refrigerated goat sperm was observed in egg yolk based media supplemented with trehalose ( $68.0 \pm 5.9$ ), not showing significant differences with the others extenders, except with the viability of the samples in BHT based media supplemented with 5% glycerol ( $38.1 \pm 4.8$ ,  $P < 0.001$ ) or trehalose ( $40.8 \pm 7.0$ ,  $P < 0.001$ ). On the other hand, also the worse results on refrigerated ram sperm were observed when the spermatozoa were preserved in BHT based medium supplemented with 5% glycerol ( $34.6 \pm 3.3$ ,  $P < 0.001$ ), showing only significant differences with the sperm viability obtained in egg yolk based media supplemented with glycerol ( $54.1 \pm 6.3$ ) and in soybean lecithin based media supplemented with trehalose ( $52.2 \pm 3.4$ ), meanwhile the observed viability in all the others refrigerated samples was similar. Nevertheless, the sperm quality motion characteristics were quite different between all the treatments in these two species, suggesting that more analysis should be made in order to explain these differences and their biological significance. Supported by INIA (RZ2009-00008-00-00), Generalitat de Catalunya (2009SGR0621 and CUR-DIUE) and FSE and Fundacion Carolina.