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Effect of the buffer system, cryoprotectant and presence of antioxidant on motile sperm population characteristics during goat sperm cryopreservation

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Our aim was to study the effect of the buffer system Tes-Tris (TEST) compared to the Tris and citric acid buffer system on sperm motion characteristics during goat sperm cryopreservation, analysed by a computer-assisted sperm system (ISAS®). Both systems were simultaneously tested in a 1% (w/v) soybean lecithin or in a 15% (v/v) powered egg yolk-based media supplemented both with 5% glycerol. Also, we assessed the effect of the inclusion of 5 mM of butylated hydroxytoluene (BHT) as an antioxidant. Briefly, fresh ejaculates from 6 Blanca de Rasquera bucks (1 year old) were collected by an artificial vagina and immediately mixed in equal quantities. The pooled semen was washed by centrifugation, and then the pellet was split into 8 equal aliquots and re-suspended in one of the 8 different extenders before freezing. In order to test the presence of separate sperm subpopulations, samples of each different treatment were taken and analysed by ISAS system after thawing. Motility data were analyzed, with the clustering procedure FASTCLUS, dividing the thawed motile sperm population in four separate subpopulations (SP), showing significant differences ($P < 0.0001$) in their motion characteristics. On the other hand, significant differences were found on the percentages of distribution of these four subpopulations (SP1 = 22.2 ± 3.7; SP2 = 5.3 ± 2.8; SP3 = 1.2 ± 0.8 and SP4 = 71.3 ± 6.6; $P < 0.0001$; mean ± SD; n = 6) between treatments. Considering that the present results are still preliminary, we could conclude that separate subpopulations of spermatozoa with different motility characteristics coexist in the thawed motile sperm population showing different behaviour in the different cryopreservation extenders, suggesting that more analysis should be tested in order to investigate the role of these subpopulations on the fertilization success. Supported by INIA (RZ2009-00008-00-00), Generalitat de Catalunya (2009SGR0621 and CUR-DIUE) and FSE.