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JOURNAL ARTICLE

Improvement of cryopreserved ram sperm heterogeneity and viability by addition of seminal plasma

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The effect of seminal plasma, as well as some seminal plasma fractions, on the heterogeneity and viability of frozen-thawed ram spermatozoa was studied. Fresh and frozen sperm samples were simultaneously assessed by a routine semen-quality assay and centrifugal countercurrent distribution (CCCD) in an aqueous two-phase system analysis. All samples used for this study were obtained from Salz rams by an artificial vagina and frozen according to the Fiser method. Sperm samples, consisting of either raw or washed semen, were frozen in the presence of either whole ram seminal plasma (RSP) or > 10 kDa seminal plasma fraction. Two control samples were considered: one diluted in Fiser's extender and an undiluted sample. Cell-membrane integrity (viability), hypoosmotic swelling (HOS) test, and sperm motility were assessed in all cases after collection, before freezing (at 5 degrees C), after thawing, and after removing the diluent before CCCD analysis. Our results show that sperm cell partition behavior was dependent on the sperm treatment and that the freezing-thawing process accounted for a clear loss of sperm heterogeneity and membrane integrity. These losses were less apparent when seminal plasma had been removed from semen samples before freezing. In addition, washed semen frozen in the presence of either > 10 kDa seminal plasma fraction or whole re-added seminal plasma maintained a higher rate of sperm heterogeneity and viability.

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