The use of L-Carnitine and Pyruvate to increase the longevity of ambient temperature liquid stored ram semen

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Cooled and cryopreserved spermatozoa have reduced longevity and viability. The highly convoluted nature of the ewe's cervix makes transcervical insemination exceedingly difficult resulting in the requirement for laparoscopic techniques to obtain acceptable fertility rates using stored spermatozoa.

We hypothesised that sperm longevity could be improved by avoiding cooling during storage, as has been demonstrated in other species. This would enable the utilisation of less invasive artificial insemination techniques, and potentially facilitate the commercial use of sex-sorted spermatozoa. Inclusion of L-carnitine in extenders supports improved mitochondrial ATP production, acts as an alternative osmolyte and stabilises cell membranes. Pyruvate provides spermatozoa with an additional energy source and acts as an antioxidant.

The aim of this study was to determine whether supplementation of ambient temperature liquid stored ram spermatozoa with L-carnitine and pyruvate increases their lifespan. Following semen collection (12 ejaculates from four rams via artificial vagina), the ejaculates were immediately extended to 4ml total volume with Biggers, Whitten and Whittingham (BWW) solution. The spermatozoa were then selected using a 90% Percoll density gradient, prior to resuspension into four treatment groups: 1) BWW; 2) BWW + pyruvate (5mM); 3) BWW + L-carnitine (100 mM); and 4) BWW + pyruvate (5 mM) + L-carnitine (100 mM). All media were osmotically balanced to 315 mOSM and 1mM of D-penicillamine was added to reduce cell-to-cell agglutination during storage. Total and progressive motility were assessed via computer-assisted sperm analysis at seven time points between 0 and 168 h. Spermatozoa were assessed for morphology using 1000X differential interference contrast microscopy, and agglutination was subjectively scored by a single assessor.

A decline in both total and progressive motility was observed across all treatments up to 120 h. At 168 h the treatments resulted in a significant increase in total motility (ANOVA, $p = 0.018$) and progressive motility ($p = 0.023$). Post hoc Tukey analysis indicated that L-carnitine (15.4 ± 2.2%) significantly improved the total motility when compared to the control (6.4 ± 2.2%; $p = 0.026$) while progressive motility was also higher in the L-carnitine treatment (13.6 ± 2.0%) when compared to the control (5.5 ± 1.9%; Tukey HSD, $p = 0.017$). Other treatments mean motilities were greater than the control, but there was no statistical significance. Cell-to-cell spermatozoa agglutination was observed from 24 h of storage despite the addition of D-penicillamine.

In conclusion, despite relatively low motility rates, L-carnitine significantly enhanced the longevity of ambient temperature stored ram spermatozoa after 7 days. Further research is required to explore optimal additive concentrations, methods to minimise agglutination and to determine the biological effects (conception rates) while managing contamination.
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