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JOURNAL ARTICLE

Effect of semen dilution on bovine sperm viability as determined by dual-DNA staining and flow cytometry

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Living and dead spermatozoa were examined for the effects of sperm concentration level on sperm viability. Semen was collected from two different bulls on each of four collection dates. A ninth bull was collected on all four collection dates as a control for effects of collection date. The ejaculates from these nine bulls were diluted to 30 x 10(6) spermatozoa/0.5 ml and then serially diluted to 20, 10, 5, or 1 x 10(6) spermatozoa/0.5 ml French straw. One-half of the straws for each dilution series was stored 24 hours at 5 degrees C, while the other half was cryopreserved. Spermatozoa were stained with SYBR-14 and propidium iodide (PI) to assess viability. Flow cytometry yielded dot plots showing three distinct sperm populations: dead red-stained spermatozoa (PI), viable green-stained spermatozoa (SYBR-14), and moribund spermatozoa that stained both red and green (doubly-stained). Populations were expressed and analyzed in terms of mean percentage of viable spermatozoa and by actual numbers of viable spermatozoa per insemination dose. The mean percentage of living spermatozoa decreased linearly with decreasing sperm concentration; whereas the decrease was parabolic when those same samples were expressed as the mean number of living spermatozoa per insemination dose. The percentage of SYBR-14-stained spermatozoa differed among concentration levels and among bulls ($P < 0.01$). There were no differences among straws from the same ejaculate. The total volume of ejaculated semen and the concentration of spermatozoa in that ejaculate were both significantly positively correlated with the percentage of SYBR-14-stained spermatozoa in that semen when it was cryopreserved and diluted to < 10 x 10(6) spermatozoa/0.5 ml. In contrast, there were no significant correlations between the initial ejaculate characteristics and the proportion of SYBR-14-stained spermatozoa in the 24-hour-stored samples at any concentration. In conclusion, the percentage of viable spermatozoa in an ejaculate significantly decreased with increasing dilution. Further, in cryopreserved samples, the percentage of living spermatozoa < 10 x 10(6) spermatozoa/0.5 ml depended on the original volume and the sperm concentration of that particular ejaculate.