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JOURNAL ARTICLE

Media and dilution procedures tested to minimize handling effects on human, rabbit, and bull sperm for computer-assisted sperm analysis (CASA)

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Proper handling of semen prior to computer-assisted sperm analysis (CASA) is critical if the analysis is to be representative of the fresh sample. The effects of diluting medium or dilution and holding time before CASA on multiple sperm characteristics were studied. Four replicates of unselected semen samples from each of eight human donors were diluted with phosphate-buffered saline (PBS)-glucose plus bovine serum albumin (BSA), with Tyrode's albumen lactate pyruvate (TALP), and with high-potassium TALP (K-TALP) to a concentration of approximately 25×10^6 (6) sperm/ml. The diluted semen was held for 0, 1, and 2 hours at approximately 30 degrees C before CASA, with little difference between the three diluents in all 12 variables measured. There was a decline of 3-6% in the proportion of motile sperm over a 2-hour period ($P < 0.05$). Donors were the largest source of differences ($P < 0.05$). Rabbit sperm (five bucks, four ejaculates per buck) were processed in a manner similar to that of the human sperm. There was a major effect of media. The average percentages of motile sperm over 2 hours in TALP, K-TALP, and PBS were 76, 42, and 29%, respectively ($P < 0.05$), with a decline of only 3% in TALP during the 2 hours. Hyperactivity and other characteristics were affected by treatment. Donors were a large source of variation. Bull semen (10 bulls, two ejaculates per bull) either was not diluted or diluted with TALP 2x or 4x and held for 0, 1, and 2 hours at 30 degrees C. It was then diluted to 25×10^6 sperm/ml with TALP. There was little change in most sperm characteristics in any treatment during the first hour, although many of the changes were statistically significant. The percentage of motile sperm in undiluted semen declined from 87% to 82% over 2 hours. Modified TALP was a suitable medium for sperm from all three species, and a simple PBS-glucose-BSA medium can be used for human sperm.

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