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## JOURNAL ARTICLE

# Comparison of motility and flow cytometric assessments of seminal quality in fresh, 24-hour extended and cryopreserved human spermatozoa

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Functional differences among fresh 24-hour extended and cryopreserved human spermatozoa were assessed using both computer-assisted semen analysis (CASA) and flow cytometry. The objective was to determine if there were interrelationships among various qualitative parameters of the fresh and treated samples when assessed by these two automated methods. Fertile donor specimens ( $n = 15$ ) were split and examined for sperm motility and curvilinear velocity using CASA within 1 hour postejaculation, after 24 hours in TEST-yolk buffer at 5 degrees C and after cryopreservation in TEST-yolk-glycerol medium. Flow cytometric analyses were performed on 24-hour extended and cryopreserved (CP) samples after fluorescent staining with rhodamine 123 to quantify mitochondrial function and carboxydimethyl fluorescein diacetate and propidium iodide to assess plasma membrane integrity. The percentages of spermatozoa with functional mitochondria and intact membranes along with the proportion of dead cells were identified and quantified by flow cytometry. Quadrant analyses of these data were used to determine the relative red and green fluorescent intensities. The initial sperm motility was correlated to the motility observed for the 24-hour stored and the CP samples. The sperm velocity of both the initial and the 24-hour extended samples was correlated to the velocity of CP samples. As for the comparison of the two automated methods for assessing seminal quality, the only sperm motion parameter that was correlated with a sperm population identified by flow cytometry (quadrant 4) was the curvilinear velocity of the sperm after 24 hours storage ( $r = 0.69$ ) and after cryopreservation ( $r = 0.74$ ). The present findings indicate that additional research is needed to determine if prefreeze analyses of donor sperm could be useful in predicting the post-thaw integrity of CP samples and, thereby, be useful in screening potential semen donors.

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Hum. Reprod., October 1, 2001; 16(10): 2109 - 2113.

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Mol. Hum. Reprod., January 1, 1999; 5(1): 29 - 37.

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