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## JOURNAL ARTICLE

# Evaluation of human sperm function after repeated freezing and thawing

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Sperm storage via freezing has been useful for men who have difficulty masturbating during assisted reproductive technology (ART) programs and before impotency caused by chemotherapy, vasectomy, and other procedures. Studies were undertaken to evaluate the extent of cryoinjury to sperm after repeated freezing and thawing. The results showed that normozoospermic and oligozoospermic sperm survived after 3 repeated freeze-thaw cycles. The inclusion of seminal plasma did not seem to protect human sperm during freezing and thawing. There were no significant differences in recovery percentages for motile, vital, and morphologically normal sperm between slow and rapid freezing methods in thaws 1, 2, and 3 of normozoospermic and oligozoospermic unwashed (u), washed (w), and washed + seminal plasma (ws) samples. However, there were significant percentage drops in the recovery of motile and vital sperm between each thaw (ie, first to second thaw, and second to third thaw) using both slow and rapid freezing for u, w, and ws samples ( $P < .01$ ). There were also no significant differences in percentage recovery of motile, vital, and morphologically normal sperm between u, w, and ws samples during thaws 1 to 3 in the normozoospermic and oligozoospermic groups. Sperm were capable of fertilizing hamster oocytes microinjected with single sperms after 3 freeze-thaw cycles as evidenced by the formation of 2 distinct pronuclei and 2 polar bodies in 22.2% and 17.2% of normozoospermic and oligozoospermic samples, respectively. The numbers of normal vital motile sperm after 3 serial freeze-thaw cycles are adequate for bringing about fertilization via intracytoplasmic sperm injection in ART programs. Thus, leftover washed sperm in laboratories that perform in vitro fertilization can be frozen, thawed, and refrozen several times without loss of the sperms' ability to fertilize. This approach has tremendous benefits for men who have difficulty producing sperm and for those with low and declining sperm counts.

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