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## JOURNAL ARTICLE

# Influence of centrifugation regimes on motility, yield, and cell associations of mouse spermatozoa

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Mouse sperm are exceptionally sensitive to mechanical forces associated with pipetting and mixing. This characteristic raised the question of the sensitivity of mouse sperm to centrifugation, a step necessary in the removal of cryoprotectants and a common component in the general manipulation of sperm suspensions for experimental purpose. Epididymal spermatozoa from ICR mice were isolated and manipulated to minimize pipetting and mixing damage. The centrifugal accelerations studied were 200, 400, 600, and 800 x g (measured with a stroboscope) for 5, 10, or 15 minutes of centrifugation time. The number of cells and the number of motile cells were counted. The percent motility and longevity, total yield, and motile yield were calculated. Centrifugation at 200 and 400 x g for short times (5 minutes) caused only a small loss in either immediate or 2.5-hour motility, but centrifugation at 600 and 800 x g for 15 minutes produced up to a fivefold loss. Low speed/short time centrifugation pelleted only about half of the cells; the others were lost when the supernatant was removed. The maximum number of motile sperm (motile yield) was obtained at intermediate centrifugal forces (approximately 400 x g for 10-12 minutes), and it is the total number of motile sperm (and not the percent motility) that is important in the use of cryopreserved sperm to regenerate cryopreserved mutant lines. Relative centrifugal force and centrifugation time exhibit reciprocity (e.g., 200 x g for 10 minutes produces similar results to 400 x g for 5 minutes). The spermatozoa must be centrifuged under carefully defined conditions to minimize the damage and to maximize the recovery of viable cells.

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