Journal of Andrology, Vol. 25, No. 3, May/June 2004

Effects of Centrifugation Before Freezing on Boar Sperm Cryosurvival

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Current protocols for boar sperm cryopreservation require the centrifugation of semen in order to separate sperm cells from the seminal plasma. This study evaluated the influence of different centrifugation regimes on both sperm recovery and yield (percentage of viable sperm with an intact acrosome relative to the initial sperm population) after centrifugation (experiment 1) as well as the influence of different centrifugation regimes on boar sperm cryosurvival (experiment 2). In both experiments, sperm-rich fractions from 3 boars were diluted, pooled, and cooled to 17°C before centrifugation. In experiment 1, the g-forces tested were 400, 800, 1600, and 2400 x g for 3 or 5 minutes, using the standard regime (800 x g for 10 minutes) as a reference. Sperm recovery (Bürker Chamber) and yield (triple fluorescent stain of PI/R123/FITC-PNA [DNA-specific fluorochrome propidium iodide/mitochondria-specific fluorochrome rhodamine-123/acrosome-specific fluorochrome fluorescein isothiocyanate-labeled peanut (Arachis hypogaea) agglutinin]) were calculated. The highest recovery and yield ($P < .05$) values were achieved using 2400 x g for 5 or 3 minutes and 1600 x g for 5 minutes, which showed no differences ($P > .05$) from the reference in terms of sperm yield. In experiment 2, cooled semen was centrifuged using 3 different regimes: C1 (2400 x g for 3 minutes), C2 (1600 x g for 5 minutes), and C3 (800 x g for 10 minutes). Pellets were diluted in lactose-egg yolk (LEY)-glycerol-Equex STM (1×10^9 cells/mL) and frozen in 0.5-mL straws. After thawing, sperm quality was assessed after 30 and 150 minutes of incubation (37°C). Centrifugation regimes C1 and C2 showed significantly ($P < .05$) higher postthaw sperm motility (assessed with a computer-assisted semen analysis system), viability (evaluated as for experiment 1), and percentage of uncapacitated sperm (assessed with a chlortetracycline assay) than did C3. In addition, C1 had the highest ($P < .05$) oocyte penetrating ability (assessed with the homologous in vitro penetration test performed with immature oocytes). Malondialdehyde production, assessed with the thiobarbituric acid reactive species test, was unaffected ($P > .05$) by the centrifugation regime used. We conclude that high g-force (2400 x g) and short centrifugation time (3 minutes) do not affect sperm recovery and yield and that, moreover, they have a positive effect on the cryosurvival of boar sperm. Therefore, we recommend the use of short-term centrifugation with a relatively high g-force (2400 x g for 3 minutes) in boar sperm cryopreservation protocol.

Key words: Cryopreservation, g-force, frozen-thawed sperm, semen quality

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