Journal of Andrology, Vol. 26, No. 5, September/October 2005

DOI: 10.2164/jandrol.05028

# Kinematic Changes During the Cryopreservation of Boar Spermatozoa

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The present study evaluates the effect that various steps of a conventional cycle of cryopreservation have on the patterns of movement exhibited by boar spermatozoa. Sperm-rich ejaculate fractions collected from 24 mature fertile boars (1 ejaculate per boar) were cryopreserved following a standard freeze-thaw procedure with 0.5-mL plastic straws. Overall sperm motility and the individual kinematic parameters of motile spermatozoa (assessed by the computer-aided sperm analysis system Sperm Class Analyzer [SCA]) were recorded in 5 steps of the cryopreservation procedure. These steps were as follows: 1) at the time that the fresh semen was extended, 2) at 17°C, after sperm concentration by centrifugation and re-extension of the pellet with lactose-egg yolk extender; 3) at 5°C, after added freezing extender; 4) at the time that thawed semen was held in a water bath at 37°C for 30 minutes; and 5) at the time that thawed semen was held in a water bath at 37°C for 150 minutes. Data from individual motile spermatozoa, defined by 7 kinematic parameters (curvilinear velocity [VCL], straight-line velocity [VSL], average path velocity [VAP], linearity [LIN], straightness [STR], mean amplitude of lateral head displacement [ALH], and beat cross frequency [BCF]), were analyzed using a pattern analysis technique (PATN) to identify and quantify populations and subpopulations of motile sperm within the semen samples. After the first cluster analysis, 3 motile sperm populations (P) were identified (P1: progressive and/or vigorous cells [90.4%], P2: poorly progressive cells [8.3%], and P3: nonprogressive cells [1.3%]). These populations remained constant ($P > .05$) throughout the 5-step cryopreservation procedure. A second PATN was carried out within the P1 sperm population, which identified 3 sperm subpopulations (sP) (eg, sP1: cells with progressive and vigorous movement [58.7%], sP2: progressive cells only [24.6%], and sP3: vigorous cells only, hyperactive-like [16.7%]). Although the relative frequency of these 3 subpopulations varied among ejaculates (boars), there was no interaction with any cryopreservation step we examined. Whereas sP1 remained constant ($P > .05$), sP2 and sP3 varied significantly ($P < .05$) through the cryopreservation procedure, with the increase in sP3 after centrifugation at 17°C and during cooling at 5°C

considered particularly relevant. In conclusion, the present study confirms the heterogeneity of sperm movement patterns in boar semen, patterns that vary through the cryopreservation procedure, especially after removal of the seminal plasma by centrifugation and subsequent extension at 17°C and after the slow cooling at 5°C, when obvious increases in hyperactivated movement appeared. The vast majority of spermatozoa, those exhibiting progressive and vigorous movement, remained constant during the cryopreservation procedure, although the proportion differed among boars.

Key words: Kinematic parameters, sperm subpopulations, pig

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