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Cryopreservation of Ram Semen Facilitates Sperm DNA Damage: Relationship Between Sperm Andrological Parameters and the Sperm Chromatin Structure Assay

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We hypothesized that cryopreservation and incubation in conditions that mimic the female genital tract following insemination increases the susceptibility of ram sperm DNA to denaturation. Ram sperm samples (n = 12) underwent the sperm chromatin structure assay (SCSA) and semen quality tests, including motility parameters, viability, and chlortetracycline fluorescence (CTC) patterns. We also assessed correlations between SCSA variables and semen quality parameters. Analyses were performed for both fresh and cryopreserved samples at 0, 3, and 20 hours of incubation in synthetic oviductal fluid (SOF; 39°C, 5% CO₂). The SCSA variables, mean alpha t (X_{α_t}) and standard deviation of alpha t (SD_{α_t}), were higher because of cryopreservation ($P < .05$, $P < .001$, respectively) after 20 hours in SOF. For both fresh and frozen spermatozoa, SCSA values (X_{α_t} , SD_{α_t} , and the percentage of cells outside the main population of α_t [%COMP $_{\alpha_t}$]) increased during incubation in SOF. Motility was negatively correlated with both SD_{α_t} and %COMP $_{\alpha_t}$, ranging from -0.39 ($P < .01$) to -0.59 ($P < .001$) for both fresh and cryopreserved semen; viability also was negatively correlated with X_{α_t} , SD_{α_t} , or %COMP $_{\alpha_t}$ (-0.36; $P < .05$, -.40 and -.46; $P < .01$, respectively) in fresh semen. The %COMP $_{\alpha_t}$ was positively correlated to the percentage of CTC pattern AR ($P < .001$) and negatively correlated to the percentages of patterns F and B (-0.33 to -0.60, $P < .05$ to $P < .001$). Variation among ejaculates within ram was observed ($P < .01$). Cryopreservation clearly facilitates DNA damage in physiological conditions. The low to moderate correlations between SCSA variables and classical semen quality parameters indicate that the SCSA provides additional information to standard tests for evaluating ram sperm quality.

Key words: Artificial insemination, semen quality, motility, chlortetracycline, viability, SCSA

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