

The Relationship between Sperm Morphology and Membrane Integrity in First and Third Ejaculates of Sexually Rested Stallions

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Evaluation of semen quality is essential for assessing the suitability of a stallion's ejaculate for cooling or freezing. Fertility evaluations in stallions are typically performed by collecting two ejaculates, one hour apart, after one week of sexual rest. The second ejaculate is thought to more closely reflect the stallion's semen quality when he is sexually active. Collecting multiple ejaculates is commonly performed as part of breeding soundness examinations in sexually rested stallions. However, this is not always completed when ejaculates are used in cooling or freezing. Semen was obtained from 10 stallions of varying fertility (10 to 79% per cycle pregnancy rate) and semen quality (37 to 78% morphologically normal sperm), presented for breeding soundness examination. The first ejaculate was obtained from the stallions after at least 7 days of sexual rest. A second ejaculate was obtained one hour later and a third ejaculate was collected the following day. Aliquots of the gel-free samples were extended to 200 million sperm/ml in EZ-mixin CST. Twelve microliters of propidium iodide and 0.7 μ L of SYBR-14 were added to 133 μ L Hepes buffer and 100 μ L of extended semen. Stained samples were incubated at 37° C for 20-30 minutes. Immediately prior to analysis, 0.5 μ L of 2% paraformaldehyde solution was added to immobilize the cells for microscopic imaging. Each microscopic field was captured under both epifluorescence and DIC using a MagnaFire® digital camera interfaced to an Olympus BX3 microscope. Captured images were evaluated side by side to compare the fluorescent staining patterns with the morphologic characteristics of individual sperm cells. Differences in means between first and third ejaculates were evaluated using paired t-tests. The percentage of morphologically normal sperm did not differ ($p > 0.1$) between first and third ejaculates. All other morphologic characteristics also occurred at a similar ($p > 0.1$) frequency in the first and third ejaculates except for the mean percentage of bent midpieces, which was higher ($p = 0.02$) in first ejaculates. Overall, the percentage of sperm with intact membranes did not differ ($p > 0.1$) between first ($58 \pm 14\%$) and third ($63 \pm 10\%$) ejaculates. Within third ejaculates, morphologically normal sperm were almost twice as likely to be membrane intact ($66 \pm 11\%$) as they were to be membrane damaged ($36 \pm 11\%$; $p = 0.003$); whereas in first ejaculates, morphologically normal sperm were just as likely to have damaged membranes ($45 \pm 17\%$) as they were to be membrane intact ($55 \pm 17\%$; $p = 0.382$). Since stresses associated with cooling or freezing could magnify membrane damage that existed prior to processing, these data support the concept of stabilizing extragonadal sperm reserves prior to cooling or freezing the semen of sexually rested stallions.

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