Sperm plasma membrane integrity during sperm extra-gonadal reserve depletion in stallions

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The sperm plasma membrane integrity during equine sperm extra-gonadal reserve (EGR) depletion has not been previously investigated. Knowing when stored sperm obtain their greatest plasma membrane quality after semen has been collected daily may improve sperm utilization. The objectives of the study were to estimate sperm output and evaluate the equine plasma membrane during sperm EGR depletion in healthy stallions. We hypothesized that the sperm plasma membrane integrity will improve during sperm EGR depletion.

Six light breed sexually rested stallions were collected daily for seven days to deplete the EGR. On collection days 1, 3, 5 and 7, a semen sample was obtained for sperm concentration and evaluation of plasma membrane integrity. A hemacytometer was used to estimate sperm concentration and the fluorescent probes SYBR-14/PI (Sperm Viability Kit®, Molecular Probes Inc., Eugene, OR) were used to evaluate the sperm plasma membrane integrity. A total of 10,000 cells, in triplicates, were analyzed for fluorescence using a flow cytometer (Accuri C6, BD Accuri Cytometers, Ann Arbor, MI). The data were examined for normality (Shapiro Wilk’s; p < 0.05) and analyzed for the effect of day on sperm output and plasma membrane integrity. The daily sperm output data were fitted into a simple linear regression equation. The plasma membrane data were analyzed using a one-way ANOVA (SAS 9.3) and where a significant effect (p < 0.05) of day was observed, Tukey’s test for multiple comparisons was applied.

The sperm concentration and plasma membrane data followed a normal distribution. The regression equation predicting sperm output (X) for stallions collected daily during sperm EGR depletion was: log (X) = 8.95 x 10^9 – 0.185 x 10^9 * (day). Intact plasma membrane percentage (mean ± SE) were 61.6 ± 3.34a, 70.7 ± 3.23b, 71.4 ± 2.28b and 69.1 ± 2.57b for day 1, day 3, day 5 and day 7, respectively. There was a significant difference in intact sperm plasma membrane between day 1 and days 3, 5 and 7, but no significant difference between days 3, 5 and 7.

We can conclude that the daily sperm output of sexually rested stallions during the sperm EGR depletion period may be predicted using the above regression equation. The plasma membrane integrity of equine sperm improved from the first collection (day 1) to the third collection (day 3); however, thereafter no improvement of sperm plasma membrane was observed. This indicates that during sperm EGR depletion, the percentage of sperm with intact plasma membrane have reached their greatest at day 3 and have the same plasma membrane quality as sperm collected after the sperm EGR depletion.

Keywords: Sperm, plasma membrane, stallion, extra-gonadal sperm reserve, sperm output