Seasonal changes in semen quality and freezability in Lusitano stallions: A flow cytometric study

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Abstract

In the present study, seasonal changes in semen quality of Lusitano stallions were evaluated to establish the best time for semen cryopreservation. Seasonal changes on testicular size, seminal characteristics and semen freezability were therefore evaluated by optical microscopy and flow cytometry. Five Lusitano stallions belonging to National Stud Farm, “Coudelaria Nacional” were used in the study. Semen was collected in four periods defined as spring (March/April), summer (June/July), autumn (September/October) and winter (December/February). Ejaculates were collected once a week for 4 weeks, and cryopreserved in 0.5-ml plastic straws. Before and after each period of semen collection, testicles of all stallions were measured. For evaluation, one straw per ejaculate, per stallion, and per season was thawed at 37 °C for 30 s and microscopic evaluation (motility) was performed. A flow cytometer (Becton Dickinson, San Jose, CA, USA) was used to evaluate sperm viability and acrosomal integrity by using the combination of fluorescent probes propidium iodide (PI) and SYBR-14 (LIVE/DEAD Sperm viability Kit (L-7011), Molecular Probes, Eugene, Oregon) for sperm viability. In addition, acrosome integrity was evaluated using the fluorescein labelled lectin from the peanut plant, Arachis hypogea (FITC-PNA, Sigma Chemical, St. Louis, MO) and propidium iodide (PI) probe. Results were evaluated by the Cell-Quest software (Becton Dickinson, San Jose, CA, USA) and statistical differences by a one-way ANOVA, followed by post hoc comparison tests. The results indicated significant seasonal differences in testicular size and sperm production (p<0.05). Ultrasonographic testicular volume was maximal in March (234.03±15.8 cm3), decreased to a minimum in October and June (203.5±17.0 cm3, 168.6±10.0 cm3, respectively) and started to increase again in January (190.8±8.8 cm3). A significant positive correlation between testicular volume and sperm production (r = 0.66; P≤ 0.01) was demonstrated, although minimum testicular volume was reached in October while minimum sperm production was registered in January. Viability in frozen-thawed semen from ejaculates collected in autumn was 37.73±2.20% and in summer 40.99±4.08%; the results differ significantly from other seasons: winter 54.26±2.10% and spring 46.71±2.32% (p<0.05). The high number of dead spermatozoa after the freezing–thawing process in autumn/winter most likely indicates that seasonally related changes in seminal plasma composition may affect semen preservation. Nevertheless, no significant differences were observed for acrosome integrity in different periods of the experiment. An inter-stallion variation was observed for testicular size and sperm production, but testicular size, besides sperm production, was positively correlated in all individual stallions. At the time of semen collection, sperm concentrations determined by photometry showed that both testis size and sperm production of housed Lusitano stallions were affected by season. Testicular size and function were maximal in the spring, but testicular volume was smallest in October, while sperm production continued to decrease till January. The results indicate that in our climatic/latitude conditions, it is preferable to freeze semen during winter/spring period.

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