

Abstract:

In the industry of bull semen freezing in artificial insemination (AI) centers, one-step and two-step semen dilution protocols are two standard and well-known methods in semen freezing process. As the freezing/thawing processes cause detrimental effects on sperm function, the addition of antioxidants can improve sperm characteristics. Hesperidin (Hesp) is an antioxidant used as the male reproductive protective agent. Therefore, the aim of this study was to investigate two different dilution methods, as well as to evaluate Hesp supplementation influence on sperm characteristics in fresh and frozen thawed semen.

Semen samples were collected from 12 Simmental bulls. Two separate examinations were conducted in, with and without Hesp supplementation groups. Statistical analysis was performed by an independent t-test, Mann Whitney test, two-way multivariate analysis of variance (MANOVA) and one-way analysis of variance (ANOVA) tests. In comparison to the one and two-step dilution protocols without Hesp supplementation, the two-step dilution showed greater cryoprotective potential. In the Hesp supplemented group, each semen sample was divided into six equal parts for experimental groups (dilution step method/ μM of Hesp). In the both one and two step dilution protocols, significant improvements were detected in semen motility parameters by Hesp administration. Also, oxidative stress status was reduced in seminal plasma of Hesp treatment groups. Interestingly, in comparison with Hesp dosage, $1\mu\text{M}$ was shown to have greater semen cryoprotective potential. In conclusion, Hesp can be used in the semen extender due to its positive influence on frozen-thawed sperm, and its combination with the two-step dilution protocol could improve frozen-thawed sperm characteristics.

Key Words: Hesperidin, Semen freezing methods, Sperm characteristics, Simmental