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Effects of post-freezing addition of relaxin on the fertility parameters of equine semen

T Nervo[1], M Poletto[1], GG Donato[1,*], A Bertero[1], L Bergamini[2], AR Elkhawagah[3]

[1] *Department of Veterinary Science, Grugliasco, Italy*
[2] *Allevamento Le Fontanette, Vigone, Italy*
[3] *Theriogenology Department, Benha University, Egypt*

The cryopreservation of equine semen, although advantageous, has critical points: the spermatozoa undergo structural and physico-chemical changes which cause alterations of flagellum, plasma membrane and cellular organelles, compromising the fertilizing potential (Freitas et al. J Equine Vet Sci. 2016; 46: 1–6). The aim of the work was to evaluate whether the post-thawing addition of relaxin, a pleiotropic hormone, had positive effects on motility, velocity, viability, membrane and DNA integrity, mitochondrial activity and apoptosis rate of the equine semen. The data were compared with a previous work, in which relaxin had been added before cryopreservation to the same semen batches (Elkhawagah et al. Anim Reprod Sci. 2020; 106351). The semen of 3 stallions of proven fertility was collected, pooled, and frozen using standard procedures, with relaxin supplementation (0, 12.5, 25, 50, 100 ng/ml). In this work, relaxin was added after thawing to the row semen at the same concentrations. Immediately after the addition of relaxin and after 15', 30', 60', 90' and 120' of incubation (37°C, CO_2 5%), sperm motility and velocity were evaluated with CASA system, viability was assessed through Hancok test and membrane integrity with Hypoosmotic Swelling test. DNA integrity, mitochondrial activity and apoptosis rate were assessed by acridine orange staining, JC-1 and annexin V-PI (flow cytometric analyses). Viability, mitochondrial activity, DNA integrity and percentage of apoptotic spermatozoa were not significantly improved after post-thawing addition of relaxin, but it seemed to exert a positive effect on the mitochondrial membrane potential and on the prevention of apoptosis, in accordance with Ferlin et al. (J Androl. 2012; 33: 474–482). The percentage of spermatozoa with intact membrane significantly increased in the samples with the post-thawing addition of 12.5, 25 and 50 ng/ml relaxin, but not in those supplemented with relaxin before freezing. The post-thawing addition of 50 ng/ml relaxin implemented and maintained sperm motility and velocity over time, particularly after 90 min of incubation, and the same results were obtained after 30 minutes of pre-freeze relaxin supplementation at 12.5 ng/ml. It was concluded that one protocol was no better than the other, but the pre-freeze addition of relaxin is more suitable for classical AI, while the post-freeze supplementation is better for deep AI, endoscopic AI or ICSI, as it also seems to activate capacitation. In the future, establishing the metabolic pathways through which relaxin acts on the male gamete will be essential to fully exploit the potential of a hormone that, only recently, has been found to be extremely important for male fertility.