

DIRECTIONAL FREEZING (HARMONY CRYOCARE – Multi Thermal Gradient 516*): A NEW TOOL FOR EQUINE SEMEN CRYOPRESERVATION

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INTRODUCTION

Success with frozen semen has not been realized in the horse industry as the pregnancy rate is reported to range from 70% to 10 %. The per-cycle pregnancy rate is for many stallions still very low (25-40%) and approximately only 30% of stallions can be considered good freezers. The limiting factors are in the freezability among individual stallions and their seminal plasma composition, extenders type used, cryoprotectants and freezing techniques such as static nitrogen vapour or computerised programmable freezing machine. Unfortunately, with the use of these systems sample temperature does not follow chamber temperature synchronously during the freezing process. This is termed supercooling and the process occurs around -6°C to -15°C. This temperature variation has detrimental effect for sperm survival (1)(2). To overcome the cryodamage of the stallion sperm cells, an automatically induced seeding and a specific directional freezing machine designed by IMT* (3) was tested in this study.

MATERIALS AND METHODS

Three semen samples from 5 trotter stallions of known fertility were split and frozen both using a conventional 0.5ml straws programmable machine (IMV) and a directional freezing technique with an MTG 516 freezing machine (IMT). This new machine is based on a series of cold copper blocks arranged in a line, with holes running through the blocks. Each block is set at two different temperatures. The extended semen, after centrifugation and resuspension, was loaded in straws and frozen with a standard curve or in Hollow TubesTM, a 9.5ml glass tube, chilled to 5°C in the fridge for 1h and frozen with MTG 516 machine. During freezing, the Hollow Tubes proceed through the holes of copper blocks at 1mm/sec, seeded at one end for 100sec in the block B (-50°C) and frozen with a linear temperature gradient till the collection chamber (-100°C). Tubes were collected at the end of copper blocks and plugged into a liquid nitrogen container. After freezing, straws were thawed at 37°C for 30 sec whereas Hollow Tubes were first at room temperature for 90 sec and then for other 30 sec at 37°C in a water flow activator. Motility was assessed by CASA; morphology, acrosome (Spermac) and membrane integrity (AO/PI) stain technique were also performed.

RESULTS AND DISCUSSION

Semen frozen with directional freezing MTG 516 showed an increase in sperm viability compared to conventional straw method. Our CASA analysis confirmed a better progressive motility with Hollow Tubes vs the same semen sample frozen in straws (50.2 vs 37.4). Moreover, viability and survival of sperm after thawing was better in the Hollow Tubes compared to straws (53.6 vs 39.5). The freeze-thaw process results in lethal damage to a high % of sperm and sub-lethal damage to the remaining cryo-susceptible spermatozoa, lowering their fertility potential. This requires insemination as close as possible to ovulation. The directional freezing with MTG 516 machine, improving sperm viability and longevity, can be speculate as an alternative method in semen freezing techniques, especially for those stallions that are considered poor freezers with the straw method. A large field insemination trial is also in progress to evaluate the fertility rate with Hollow Tubes compared to the straws method.

REFERENCES

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