COMPARISON OF SLOW AND RAPID FREEZING FOR LONG TERM STORAGE OF FREEZE-DRY RAM SPERMATOZOA

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Semen lyophilization is an interesting technique that might be a cheap alternative to long-term storage under liquid nitrogen. The first significant result of this method was achieved by Wakayama and Yanagimachi in the 1998 [1] demonstrating for the first time the birth of healthy offspring from epididymal freeze-dried (mouse) spermatozoa. From this work on, the most used approach for lyophilisation is that of deep-freezing, that is directly immersing the semen sample into liquid nitrogen before vacuum drying. Recently we have shown that it is possible to establish a "dry" bank of ejaculated and epidydimal freeze-dried ram spermatozoa [2, 3]. In order to improve and make the technique more reliable, here we focused on the freezing phase, comparing two different protocols: i) Fast-freezing, where the semen is plunged directly into liquid nitrogen (LN group); ii) Slow-freezing, where the sample is progressively cooled to a final temperature of -50°C (SL group). Briefly, for the preparation of the LN group sample the protocol reported in [2] was followed, while for the SL group the semen was frozen with a freezing rate of 1°C/min until -50°C degrees, when the sample was placed inside the lyophilizer. Dry spermatozoa from both groups was used for Intracytoplasmic Sperm Injection (ICSI) and the embryo development was evaluated at 24h (2-Cells stage) and 7 days (expanded blastocyst) after fertilization. At 24h post fertilization the SL-group showed a higher number of cleaved embryos than LN-group (42/100 (42%) versus 19/75 (25.3%), *P*=0.0253, SL and LN respectively). At 7 days after fertilization the blastocyst rate in SL-group was higher (7/100 (7%)) than in LN-group (2/75 (2.7%)), although not statistically different. Our data shows that lyophilisation can be conveniently achieved in ram spermatozoa without previous freezing in liquid nitrogen, thus simplifying the procedure. This data supports the idea that lyophilisation might be a valuable and cheaper alternative to liquid nitrogen for long-term storage of ram semen.

[1] Wakayama T and Yanagimachi R. Development of normal mice from oocytes injected with freeze-dried spermatozoa. Nat Biotechnol. 16:639-41, 1998. [2] Anzalone DA et al. Freeze-dried spermatozoa: An alternative biobanking option for endangered species. Anim Reprod Sci. 190:85-93, 2018. [3] Palazzese L et al. DNA fragmentation in epididymal freeze-dried ram spermatozoa impairs embryo development. J Reprod Dev. 64:393-400, 2018.