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Cryopreservation of feline oocytes by vitrification using commercial kits and slush nitrogen technique

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Cryopreservation of oocytes is still considered an experimental technique in felids, although it is highly demanded to be used for assisted reproduction in conservation breeding programmes. Despite that vitrified oocytes have shown to be able to produce live kittens, results are not yet satisfactory due to very low oocyte survival rates. The post-thaw oocyte competence to cleave after fertilization is reported between 14 to 25%, while in cattle or human this percentage ranges from 60 to 86%. The aim of the present study was to compare two different commercially available vitrification kits with our method (IZW), established for cryopreservation of feline oocytes.[1] Commercial kits (VitKit®, IrvineScientific; Kitazato®, Kitazato BioPharma) offer an easy-handling and standardized approach, but species-specific characteristics may reduce the freezing success. The IZW protocol presents a 3-step vitrification method with a mixture of ethylene glycol, dimethyl sulfoxide, fetal calf serum, trehalose and ficoll PM-70. In addition, we applied ultra-rapid freezing by slush nitrogen in order to improve cat oocyte survival, since it provides until ten times higher cooling rates than those obtained with the regular vitrification. Cumulus oocytes complexes (COCs) were collected from domestic cat ovaries by slicing. Only high quality COCs were chosen for vitrification using the Cryotop technique. In the first experiment, all three different vitrification methods were compared. After thawing, COCs were matured and fertilized by ICSI to assess their developmental competence. In a second experiment, ultra-rapid vitrification in slush nitrogen produced with VitMaster® device was applied for the two best vitrification protocols. The VitKit® (n=89) showed the lowest maturation rate obtained after thawing (10.1%). Furthermore, a significant difference in maturation rate of oocytes was found also between Kitazato® Kit (38.7%, n = 137) and IZW protocol (24.5%, n = 143). The cleavage after ICSI of matured oocytes (20.8% and 28.6%, respectively) and the morula rate (18.2% and 22.5%, respectively), however, did not reveal any significant difference between the two methods. Ultra-rapid vitrification, did not result in any improvement of oocytes cryopreservation. Thus, maturation rate of the oocytes cryopreserved by IZW method with slush nitrogen (n=114) decreased until 6.1%, and none of these oocytes were competent to cleave. In the case of Kitazato® (n=62), only 17.7% were able to undergo maturation and cleavage rate dropped to 18.2%, not reaching morula stage. Our data demonstrate that domestic cat oocytes can be vitrified by our IZW protocol as well as by the commercial Kitazato® Kit with a comparable outcome, however the results on post-thaw embryonic development are still insufficient. In contrast to our expectation and data from other species, ultra-rapid freezing by slush nitrogen was not supportive for post-thaw survival of feline oocytes. Further studies and, perhaps, other innovative approaches are required to achieve better post-thaw survival of feline oocytes.