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Development of a novel 3D approach for cat oocyte cryopreservation

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Introduction and aim. Three-dimensional (3D) systems were established to provide cells with an in vivo-like environment, and this prompted the use of 3D oocyte culture. The novelty of this work, instead, is to exploit the biophysical characteristic of a 3D scaffold (alginate microcapsules) for reducing stress and manipulation during vitrification of cat immature oocytes, providing a matrix that is employable for downstream applications, such as in vitro maturation (IVM). To achieve this goal, preliminary assessments were required, as the ideal size of alginate microcapsules, their response to a standard vitrification protocol, and the viability and nuclear status of cat oocytes after encapsulation in different concentrations of alginate and 24 hours IVM.

Materials and methods. Size of cat cumulus-oocytes complexes (COCs) after IVM-induced cumulus expansion and the width of the vitrification device (Cryotop) were measured with ZEN microscopy software v.6.3 to define microcapsule size. Then, alginate microcapsules were generatedby manually dropping sodium alginate (0.5%, 1%) into $CaCl_2$ 100 mM [1] and vitrified according to [2] with standard equilibration in cryoprotectant solutions, or following equilibration in plain Medium 199. Ice formation (i.e., vitrification failure) was evaluated visually as capsule opacifying, while translucent capsules were deemed ice-free. Immature grade I oocytes were collected from 44 ovaries. Control IVM and 3D IVM in 0.5% and 1% alginate microcapsules were performed in standard medium, as in [3]. After IVM, oocytes were denuded, stained with fluorescein diacetate-propidium iodide to assess viability, and then stained with Hoechst to assess the nuclear status. At least 3 replicates were performed for each experiment. Data were analyzed by chi-square or Fisher's exact test; $p<0.05$.

Results. Based on average diameter of COCs (0.46±0.21 mm; n=46) and Cryotops width (1.01±0.01 mm), capsule size was set around 1 mm, as in (1). Vitrification was successfully ice-free in 100% of microcapsules (n=29) equilibrated in cryoprotectant solutions against 0% of those exposed to plain medium (n=29, $p< 0.00001$). No difference ($p=0.9$) was found in viability of control (91%, n=47) and 3D matured oocytes (96% for both 0.5% and 1% alginate, n=53 and n=49, respectively). Full maturation rates were significantly higher for oocytes matured in 1% alginate (76%) vs. control (55%, $p=0.04$). No differences were found for 0.5% alginate (68% maturation, $p=0.4$).

Conclusions. Alginate microcapsules 1% sustained cat oocyte viability and improved their maturation, besides being suitable for obtaining the typical amorphous state of vitrification. The determination of cryoprotectant permeation in encapsulated oocytes is one of the next steps, before the evaluation of oocyte developmental competence following 3D cryopreservation.

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