Enriched microcapsules for the culture and preservation of domestic cat cumulus-oocyte complexes

Martina Colombo, Beatrice Anna Riganti, Alessandra Mascaro, Jasmine Fusi, Gaia Cecilia Luvoni
Dip. di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Lodi

Alginate microcapsules have proved to be a suitable microenvironment for the in vitro maturation (IVM) of cumulus-oocyte complexes (COCs) in different species. By more closely replicating the native ovarian milieu, these three-dimensional (3D) systems offer significant advantages for oocyte maturation and developmental competence compared with traditional two-dimensional (2D) approaches. More recently, alginate microcapsules have also successfully been employed for the cryopreservation of COCs. The addition of extracellular matrix components could further increase the biomimicry of these systems. Thus, the aim of this study was to assess the effects of hyaluronic acid (HA) and collagen type I (CI) as functional enrichments for domestic cat COCs IVM (Exp. I) and cryopreservation (Exp. II).
Immature grade I COCs were collected from spaying-derived ovaries. In Exp. I, COCs were cultured in IVM medium for 24 h in 2D or 3D conditions. For the latter, COCs were encapsulated in 1% alginate (3D) or in 1% alginate enriched with 2 mg/mL HA (3D+HA) or 0.04 mg/mL CI (3D+CI) [1]. In Exp. II, encapsulated (3D) or non-encapsulated (2D) COCs were slow-frozen following a standard protocol [2]. After thawing, COCs were in vitro matured as above, using a group of fresh COCs as a control. After IVM, all COCs were denuded, stained with fluorescein diacetate for viability, ReadyProbes™ Reagent for actin distribution and Hoechst for nuclear status. At least 3 replicates were performed for each experiment. Data were analyzed by Fisher's exact test, $p<0.05$.
Results of Exp. I (n=36-41 COCs/group) showed no differences in viability (89-97%, p=0.36) and normal actin distribution (88-92%, p=0.71). Full maturation rates were maintained in 3D+CI (56%), but lowered (p=0.04) by the addition of HA (41%) compared to 2D (63%). Therefore, CI was chosen for Exp. II (n=46-53 COCs/group). No differences were found in maturation rates among cryopreserved groups (2D, 3D and 3D+CI, 31-33%, p=1), with fresh control COCs showing a higher rate (66%, p=0.001). Although a similar trend was observed for viability (p=0.28) and actin distribution (p=0.38) among cryopreserved groups, 3D+CI showed viability (69%) and normal actin distribution (71%) similar to those of control fresh oocytes (83% and 81%, p=0.16 and p=0.35, respectively), while 3D did not (57% and 62%, p=0.007 and p=0.004).
In conclusion, while CI did not influence the outcomes of the IVM of fresh cat COCs, its use during 3D cryopreservation could help mitigate freezing injuries. Further studies will help clarify whether preservation of immature oocytes in enriched-3D matrices could enhance developmental competence and indicate if this strategy can be applied for felid germplasm banking.
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[1] Mastrorocco et al. Hyaluronic acid/collagen I-enriched 3D-microbeads fabrication and matching to ovine oocyte IVM, Proceedings of 1st TIAR, p. 143-146, 2024
[2] Luvoni et al. Embryo development in vitro of cat oocytes cryopreserved at different maturation stages, Theriogenology, 53:1529-40, 2000