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Effect of a three-dimensional co-culture system on feline oocyte competence to develop into an embryo.

Maria Giorgia Morselli¹, Gaia Cecilia Luvoni², Pierre Comizzoli³

¹Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare; Università degli Studi di Milano, Italy; ²Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC, USA.
mariagiorgia.morselli@unimi.it

Three-dimensional (3D) in vitro culture has been developed to improve the full competence of mammalian oocytes. These culture systems mimic the in vivo spatial arrangement and physiological conditions for the oocytes. In addition, the co-culture with companion cells, such as cumulus-denuded oocytes (CDOs) or cumulus cells (CCs) clumps, has been shown to improve the oocyte maturation and subsequent embryo development in different species, including domestic cat [1,2,3]. The aim of this study was to investigate the effect of an enriched culture system (3D barium alginate microcapsules associated with companion CDOs) on the in vitro embryo development of feline cumulus-oocyte complexes (COCs). Grade I COCs (n=545; 8 to 12 replicates) were collected from adult ovaries and 187 were mechanically deprived of CCs to obtain the CDOs. The COCs were co-cultured with CDOs in barium alginate (Sigma Chemical Co., MO, USA) microcapsules (3D system) in Quinn’s Advantage Protein Plus Blastocyst medium (SBP, SAGE® In Vitro Fertilization, Trumbull, Connecticut, USA) with 75 UI FSH + 75 UI LH (Menogon®, Ferring Pharmaceuticals, Switzerland), 10 ng/ml of epidermal growth factor (EGF), antibiotics (AB) and 0.6 mM cysteine, in a controlled atmosphere (38.5°C and 5% CO2 in air) for 24 h. Control groups of COCs alone were cultured separately. For in vitro fertilization, chilled epididymal spermatozoa were selected by swim-up treatment in SBP and the oocytes were inseminated with 0.75-1 x 10⁶ motile spermatozoa/ml. At 18-24 h post-insemination, presumptive zygotes were in vitro cultured for 7 days in 3D microcapsules in SBP with 5% of FCS and AB (National Institutes of Health, Bethesda, MD, USA). The oocyte meiotic progression after IVM and embryo stages at the end of culture were determined by fixation and staining with bis-benzimide (Hoechst 33342; Sigma). Data were analyzed by Chi-square test (p<0.05). The meiosis resumption of COCs (in co-culture: 86.1% and cultured alone: 88.5%) was significantly higher than that of CDOs (40.2%; p<0.00001). The COCs cultured separately showed the highest full maturational (TI-MII) rates (p<0.00001) compared to those of COCs co-cultured with CDOs (74.2% vs 37.5%) and of CDOs themselves (74.2% vs 12.5%). Inversely, the proportions of late embryos stages (total n. morulae and blastocysts/total n. cleaved embryos) of COCs co-cultured with CDOs were higher than those of COCs cultured separately (77.6% vs 53.8%), and no difference was found between CDOs and COCs control (58.8% vs 53.8%; p=0.05). The 3D barium alginate microcapsules in association with companion CDOs were a suitable enriched culture system to improve the developmental competence of domestic cat COCs. The presence of denuded oocytes and related secreted factors supported the achievement of COCs cytoplasmic maturation, essential for further embryo development. Moreover, the CDOs themselves, known as low competence gametes, also benefited from this condition, as their late embryo stages were similar to those of COCs control.