

Bioactive microenvironments for gonadal cell development in small animals.

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The *in vitro* development of gonadal cells into mature germ cells may enable the generation of progeny from a broader range of precursor populations, including early-stage gametes and stem cells. *In vitro* gametogenesis holds significant promise for fertility preservation and biodiversity conservation, particularly in prepubertal individuals and endangered species.

However, its success is still limited. Complete *in vitro* growth of follicles until the oocyte metaphase II stage has been achieved in rodents and humans [1], while *in vitro* spermatogenesis has only been obtained in murine models [2]. Mimicking gametogenesis *in vitro* is very challenging, and recreating *in vivo*-like conditions seems key to a successful outcome. The ovarian and testicular niches provide nutrients and signaling and support the survival and development of somatic and germ cells. Cells can be cultured *in situ* (i.e., within the surrounding tissue) or *ex situ* (i.e., isolated), using traditional or three-dimensional (3D) culture systems, including hydrogels, organoids, and 3D-printed or decellularized biomimetic scaffolds. Culture enrichments such as companion cells or extracellular matrix compounds can be added to the *in vitro* system with the final goal of creating a bioactive microenvironment that can promote growth and lead to the development of mature, functional gametes.

Only some of these systems have been tested in small animals, whose peculiar reproductive physiology may limit the efficacy of approaches developed in other species. The achievement of *in vitro* gametogenesis would be desirable in dogs and cats both for the species *per se* and for their value as comparative models. For female gametes, bioactive microenvironments have been applied to ovarian cortex, isolated follicles and immature oocytes. Several systems were investigated, including air-liquid interface, hydrogel encapsulation, 3D co-cultures, and microfluidic platforms. So far, the short-term hypertonic exposure of alginate-encapsulated feline secondary follicles led to full meiotic competence of enclosed oocytes [3]. In dogs, this endeavor is more challenging due to the limited *in vitro* maturation ability of canine oocytes. For male gametes of both species, similar culture environments have been used for isolated cells and testicular fragments, generally supporting the maintenance and proliferation of the spermatogonial populations, but not their progression to mature gametes, unless transplantation was also employed.

Despite the challenges, continuous progress is being made in the study of bioactive microenvironments for gonadal cell culture in dogs and cats. Tailoring the *in vitro* niche to species-specific and organ-specific requirements could support the preservation of valuable genetic pools and drive the search for efficient culture systems for small animal cells.

[1] Telfer EE, Grosbois J, Odey YL, et al. Making a good egg: human oocyte health, aging, and *in vitro* development. *Physiol Rev* 2023;103(4):2623–77.

[2] Sato T, Katagiri K, Gohbara A, et al. *In vitro* production of functional sperm in cultured neonatal mouse testes. *Nature* 2011;471:504–7.

[3] Songsasen N, Thongkittidilok C, Yamamizu K, et al. Short-term hypertonic exposure enhances *in vitro* follicle growth and meiotic competence of enclosed oocytes while modestly affecting mRNA expression of aquaporin and steroidogenic genes in the domestic cat model. *Theriogenology* 2017;90:228–36.