ABSTRACTS

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Domestic dog and cat models for understanding cellular factors that regulate in vitro follicle development, including potential application to wild carnivores

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One major challenge to applying in vitro embryo production to preserving fertility of valuable females is related to the rarity of, and accessibility to, mature oocytes capable of fertilization. Within the ovary, there are hundreds of thousands of immature follicles containing oocytes that are never ovulated or fertilized (1). The ability to grow these immature primordial and preantral follicles to a mature stage containing fertilizable eggs has enormous potential for rescuing and protecting genetic diversity of valuable genotypes and species, including endangered wildlife. This approach would allow ‘genomic rescue’ and be especially valuable for the genetic management of rare species where there is a prevalence of females who are under-represented in the population or die before reaching puberty. To date, live mice have been produced from oocytes recovered from in vitro grown follicles (2). There have been few attempts to apply these strategies to larger-sized mammals, including canids and felids (3).

Our laboratory has studied the dog and cat model for decades to generate fundamental data on these two important companion animals, and to produce knowledge and approaches applicable to wildlife counterparts. This is important because 10 of 36 extant canids and 25 of 37 felids (4) are listed formally as threatened by extinction. Recently, we have studied the factors regulating the survival of ovarian tissue in vitro, and determined that it is possible to sustain living dog and cat ovarian tissue that retains primordial follicle viability for up to 2 weeks. An advantage of simultaneously studying both species is the recognition of remarkable species-specificity in requirements for the in vitro microenvironment. As one example, -minimum essential medium (MEM) maintains viability of dog ovarian follicles in culture, but not in the cat. By contrast, cat follicle viability is retained for up to 2 weeks in MEM, which has a much lower amino acid content than -MEM. To further illustrate, epidermal growth factor (EGF) has no effect on cat follicle survival (P > 0.05) as presence or absence results in 50 versus 65% survival, respectively (P > 0.05) after 2 weeks. However, EGF enhances (P < 0.05) dog follicle viability (55%) compared to those grown in its absence (i.e., 35%). There also is evidence that systems being developed in the domestic models are applicable to wild counterparts. For example, the dog microenvironment has been used to retain ~30% follicle viability after 9 days of culture in the maned wolf (Chrysocyon brachyurus). Another contemporary study has involved examining a three-dimensional culture system using alginate hydrogel for isolated, preantral dog follicles. Our findings indicate that physical and hormonal microenvironments significantly affect follicle growth and steroidogenesis (5). Specifically, follicles encapsulated in 0.5% alginate grow faster than those in 1.5% concentration (5). Furthermore, follicle stimulating hormone (FSH) has been essential for in vitro folliculogenesis, with an absence in this gonadotropin associated with retarded follicle growth and steroidogenesis (5). Luteinizing hormone promotes (P < 0.05) in vitro follicle growth in the absence of FSH and enhances (P < 0.05) steroid production. We have also discovered that type and concentration of protein supplementation affect follicle growth and morphology of cultured oocytes. Specifically, culturing preantral follicles in the presence of 10% canine serum (CS) has been detrimental to follicle development. Although growth rates of follicles cultured in a protein-free medium, 10 mg/ml fetuin, 1% CS and 3% CS are comparable, oocyte morphology has differed among groups. Specifically, eggs incubated in medium containing CS have retained the characteristic, normative dark cytoplasm compared to the pallid appearance of those exposed to other microenvironments. Therefore, we suspect that the CS is supporting lipid metabolism and/or development of dog oocytes in vitro.

In summary, we are addressing many mysteries associated with in vitro follicle growth in the dog and cat. It is clear that the mechanisms driving success are much different from the traditionally studied mouse model and even between these two carnivore species. This finding itself reinforces the need for more comparative studies between species (6) and the investigation of larger sized models, especially for those keen to adapt this technology to fertility preservation in women. Although we have data indicating the potential of growing dog and cat early stage follicles in vitro, actual practical application will require culture systems that can retain viability for up to 8 weeks to allow recovering mature-stage, fertilizable oocytes. Lastly, a high priority is to begin to study the fundamental traits of the dog and cat egg itself from within the follicle, especially the kinetics of its structural and developmental competence.

(4) IUCN 2011 The IUCN Red List of Threatened Species. www.redlist.org