Ovarian Stromal Cell Conditioned Media Improves Survival in Pre- and Early Antral Cat Follicles

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Ovarian stromal cells act as crucial support and regulators for *in vivo* folliculogenesis; however, less is known about their effect on *in vitro* grown follicles. The objective of this study was to investigate the impact of ovarian stromal cell co-culture or conditioned medium (CM) on survival and development of cat pre-, early, and antral follicles *in vitro*.

Ovaries were obtained from cats older than 6 months (n = 3), then enzymatically digested to release stromal cells. The ovarian stromal cells were allowed to grow to confluency in a T75 flask, before being cryopreserved for long term storage in liquid nitrogen. Cells were thawed one week prior to follicular culture onset, and passaged once before CM collection. CM was subsequently removed 24 - 48 hours after feeding, and stored at -80°C until used. Ovarian follicles were mechanically isolated from cats older than 6 six months (n = 23 cats, 155 follicles), encapsulated in 0.5% alginate hydrogel, and cultured for 13 days in Endothelial Cell Growth Medium. The isolated follicles were then divided into five treatment groups (control, ovarian stromal cell co-culture, 20% CM, 50% CM, and 100% CM), and classified based on initial diameter as preantral (224.4 $\pm$ 4.7 μm), early antral (394.8 $\pm$ 7.4 μm), or antral (592.2 $\pm$ 18.8 μm). The survival and growth of isolated follicles were then evaluated on Day 0, 4, 6, 8, 11 and

13. At the culture period endpoint, follicles were assessed via RTPCR for expression of *CYP19A*, *FSHR*, and *GDP9* to further quantify development. Statistical analysis was done in R software.

Follicles in 100% CM had higher survival up to Day 11 of culture as compared to other treatment groups (Cox proportional hazards model, $p \leq 0.01$). Initial stage also influenced survival, with antral follicle survival significantly lower than that of pre- and early antral follicles ($p \leq 0.0001$). However, no differences in growth were detected across the treatment groups, nor across initial size classifications (Kruskal-Wallis test, $p > 0.05$). Post culture RTPCR analysis of the three selected genes showed *CYP19A* was upregulated in 50% CM follicles compared to the control (ANOVA, $p \leq 0.05$). However, there were no differences in *CYP19A* expression between the control and other treatment groups, or in *GDF9* and *FSHR* expression among culture groups ($p > 0.05$). In summary, the findings demonstrated that conditioned medium collected from primary culture of ovarian stromal cells improves *in vitro* survival and modulates *CYP19A* expression of isolated cat follicles. Further research to identify paracrine factors present in conditioned medium will elucidate the roles of ovarian stromal cells pertaining to follicle survival during *in vitro* folliculogenesis.