ABSTRACTS

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In vitro survival of preantral follicles recovered from queens at different stages of estrous cycle and cultured with IGF-1

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OBJECTIVE AND METHODS: In vitro growth of preantral follicles may supply high numbers of competent oocytes that can be destined to in vitro embryo production. However, optimal culture systems that support follicular and oocyte development in vitro have not yet been defined. Ovarian follicular development is regulated mainly by endocrine, autocrine, and paracrine factors and follicles are exposed to specific hormonal environments during the different stages of the estrous cycle. The intraovarian insulin-like growth factor-I (IGF-1) regulates autocrine, and paracrine factors and follicles are exposed to specific hormonal environments during the different stages of the estrous cycle. The importance of IGF-1 in follicular development has been previously described (1). Feline preantral follicles have been previously cultured in vitro (for review 2) and it has been suggested that IGF-1 enhances oocyte metabolism (3). However, no information are available on the response of preantral follicles recovered in different phases of the estrous cycle to cultural conditions. Thus, the aim of this study was to investigate the in vitro survival of preantral follicles recovered from ovaries of queens in follicular or luteal phase of the estrous cycle and cultured in presence of IGF-1.

Twelve queens were housed with 12 hours of light and submitted to estrous induction with IM injection of 100 UI eCG (Novormon®- Intervet) and 100 UI hCG (Vetecor®- Hertape Calier) 82 hours later (4). Six queens were spayed 96 h after eCG administration (follicular phase), the others 36 h after the hCG injection (luteal phase). Estrous phases were confirmed by vaginal cytology prior to the surgical procedure and macroscopic evaluation of ovarian structures after excision. Preantral follicles surrounded by complete basal membrane and containing more than one layer of granulosa cells were retrieved from excised ovaries and selected as previously described (5). A total of 72 follicles were cultured for 6 days at 38.5 °C and 5% CO2 in air in Minimum Essential Medium (Sigma Chemical Co., USA) with IGF-1 100 ng/ml (Sigma) or without (control). Before and after culture, follicular diameter was recorded and follicular viability was assessed by fluorescein diacetate (FDA, Sigma) staining. Increase (%) in diameter was analyzed by Tukey’s and Fisher’s test, viability rates by Chi-square test (P<0.05).

RESULTS: After 6 days of culture, preantral follicles retrieved during follicular phase showed a significant increase in the size and a higher viability rate than those retrieved in the luteal phase of the estrous cycle (18.8% vs.11.5%; P= 0.0001 and 75% vs. 62.5%; P=0.004). However, when IGF-1 was added to the culture medium, follicles retrieved in follicular or luteal phase showed similar increase in diameter (14.9% vs.13.4%; P>0.05) and viability (73.8% vs. 76%; P>0.05). Regardless of the stage of the estrous cycle, overall results showed that the increase in diameter was not different in follicles cultured with or without IGF-1 (14.6% vs. 15.8%; P>0.05), but follicular survival was enhanced when IGF-1 was added to the culture medium (75% vs. 69.4%; P=0.0001).

CONCLUSION: Present data suggest that in vitro survival of preantral follicles is affected by the estrous stage of the donor and IGF-1 improves survival of follicles retrieved in luteal phase of the estrous cycle. Thus, the hormonal environment of the follicles within the ovary might impact their potential development when isolated and cultured. Further investigations on growth factors are needed to evaluate their effect on follicles with reduced in vitro developmental capability.