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Efficacy of different methods of isolation on growth of ovine preantral follicles

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Mammalian ovaries contain an elevated number of preantral follicles that are not easily recruitable for *in vitro* culture up to the terminal stage of differentiation. Attempts have been made to obtain complete development of mammalian preantral follicles *in vitro*. In the present study the efficacy of different methods for isolation of preantral follicles from ovine ovaries was examined. Preantral follicles were isolated by mechanical dissection of preantral follicle methods, microdissection, enzymatic digestion (Trypsin digestion 1 and 0.5% for 10 or 5 mins), collagenase digestion (0.5 and 0.25% for 2 and 5 mins). Both small pre-antral follicles (SPFs, 100–250 μm) and large pre-antral follicles (LPFs, 250–450 μm) were isolated. The trypsin (1%) digestion for 5 minutes resulted in maximum isolation of total (SPFs: 24.0 ± 3.1 LPFs: 13.2 ± 2.4) and viable preantral (SPFs: 18.0 ± 2.5 LPFs: 10.4 ± 2.0) follicles. The preantral follicles were cultured *in vitro* for 8 (short term) and 28 days (long term culture), respectively, and examined for their growth, survival and antrum formation rates and growth rates of oocytes in cultured preantral follicles. The *in vitro* growth, survival and antrum formation rates of preantral follicles and growth rates of oocytes in cultured preantral follicles were significantly ($p < 0.05$) higher when isolated by trypsin digestion (1% for 5 mins) or mechanical isolation compared to other isolation methods.

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