ACCELERATION OF FOLLICULAR DEVELOPMENT IN VITRO: MORPHOLOGICAL AND MOLECULAR EVIDENCE

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Abstract Body

In the context of fertility preservation, ovarian tissue cryopreservation followed by culture of isolated follicles is a promising approach. It is then crucial to develop a culture system preserving the integrity of the oocyte-granulosa-theca interactions while allowing an optimal follicular maturation in order to produce *in vitro* an oocyte of good quality for subsequent fertilization. Therefore, appropriate parameters must be determined to evaluate the best conditions for follicular *in vitro* development.In prepubertal lamb, after isolation from ovarian cortex strips, preantral follicles were cultured individually in microdrops under oil in aMEM+ medium supplemented with insulin. Then, we compared follicles cultured for 1, 6, 13 and 20 days to follicles of similar sizes developed *in vivo*. The expression of 36 genes was analyzed using the qPCR BioMark™ System (n=1389 follicles). In parallel, the morphology of 111 follicles of the same stages was analyzed, including the determination of the follicle and oocyte diameters, the number of follicular cells and the antrum volume.In this culture system, the follicles developed from the preantral (180-240µm) until the antral stage (up to 800µm) over a 20 days period. Follicular development involved oocyte growth (x1.3), follicular cell proliferation (x20), and the appearance and development of the antrum (from day 6). However, through comparison with *in vivo* development, quantitative differences in growth parameters were found. Particularly, oocyte growth and antrum development were significantly reduced, but cell proliferation was enhanced in cultured follicles ($p<0.001$). These differences in follicular morphogenesis were accompanied with changes in the expression of genes involved in somatic cell proliferation (*CCND2, CDKN1A*) and differentiation (*CYP19A1, AMH, FSHR, LHCGR),* together with an impairment of the expression of oocyte-specific markers such as *BMP15*.In conclusion, despite a harmonious follicle development in culture, the observed patterns of gene expression suggested an early maturation of the cultured follicles.