

## Publication

### A novel three-dimensional culture system allows prolonged culture of functional human granulosa cells and mimics the ovarian environment

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The development of techniques allowing the growth of primordial follicles to mature follicles in vitro has much potential for both reproductive medicine and developmental research. However, human primordial and preantral follicles fail to grow after isolation from the surrounding ovarian stroma. Granulosa cells, which normally undergo apoptosis after ovulation, contain a subpopulation of ovarian follicular cells remaining viable in vitro over prolonged periods when cultured in the presence of leukemia-inhibiting factor. However, when cultured as monolayers, they progressively lose all their characteristics, such as follicle-stimulating hormone receptor and cytochrome P450-aromatase. Here, we describe a three-dimensional culture system containing type I collagen, which, together with leukemia-inhibiting factor, allowed the survival and growth of a subpopulation of granulosa cells isolated from mature ovarian follicles and supported them to proliferate into spherical structures exhibiting steroidogenic capacity, as demonstrated by P450-aromatase and 3 $\beta$ -hydroxysteroid dehydrogenase. After transplantation into the ovaries of immunodeficient mice, these cells became localized preferentially within antral follicles and the prolonged expression of follicle-stimulating hormone receptor was confirmed as well. With this optimization of the culture conditions, an environment was created, which acts as a niche closely mimicking the development of early ovarian follicles in vitro.

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