

Mouse oocyte development - Methods and Protocols

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The Springer Protocols series “Methods in Molecular Biology” has published its 1818th volume which is entirely devoted to the development of the female gamete: the oocyte.

Many other volumes like *Mammalian oocyte regulation* (Homer H.A., ed., 2013), *Oocyte biology in fertility preservation* (Kim S.S., ed., 2013), *The fish oocyte* (Babin, P.J., Cerdà J., Lubzens E., eds., 2007), *The future of oocyte* (Eppig J., Hegele-Hartung C., eds., 2002), *Oocyte growth and maturation* (Dettlaff T., Vassetzky S.G., eds, 1988), just to cite a few, have been published over the years reflecting the need to face this topic, the oogenesis, from different points of view.

This last volume is divided into seven-teen chapters describing the methods to obtain *in vitro* growth of follicle cells, *in vitro* maturation of mouse oocytes, pronuclear transfer, transcriptome profiling and to perform immunocytochemical techniques to stain and follow organelles dynamics and several markers important for a correct oocyte growth and maturation.

Being involved in the study of mouse and human oocytes and in the molecules/mechanisms behind the oocyte ability to resume meiosis and complete the proper embryo development past the 2/4-cell stage, I strongly suggest the reader to go through the pages of this book. If you are moved by the uncontrollable curiosity to finely dissect the molecular mechanisms and cytological events underlying the ability of the oocyte to resume meiosis, here you can find the correct hints to set up the right protocols for your experiments.

The first three chapters, in particular,

describe the methods to grow granulosa cells *in vitro* and to culture both mouse and human oocytes. Images and pictures, together with detailed protocols are here presented. Pronuclear transfer and *in vitro* fertilization are well detailed in the following chapters enriched with drawings (chapter five) of both male and female mouse reproductive tracts to help the beginners.

Chapters six and seven, written by experts in the oocyte field, detail the molecular constitution of the developing oocytes (*Profiling maternal mRNA translation during oocyte development* by Joao Sousa Martins and Marco Conti and *transcriptome profiling of single mouse oocytes* by Edith Heard and colleagues) guiding the reader, step by step, in the procedures presented from oocytes collection to cell lysis for library preparation and sequencing.

Experienced (and not) histo- and cytochemists dealing with immunofluorescence techniques will be interested in the following two chapters. This approach, critical to determine the protein function, regulation, and temporal and spatial location is presented to detect subcellular structures critical to oocyte maturation (chapter eight) and to study kinetochores (chapter nine). Sample preparation, fixation, permeabilization and antibody application are explained in a precise and easily reproducible way.

The triple-color live imaging technique detailed in chapter ten is very interesting because enables the simultaneous spatiotemporal mapping of three different components of the spindle and chromosomes thanks to the microinjection of RNAs encoding proteins tagged with green and red fluorescent proteins and the visualization of microtubules with the fluorogenic far-red SiR tubulin.

The large size of the oocytes allows the study of several peculiar aspects of the oocytes biology thanks to their manipulation: this technical aspect is not suitable for cells with smaller dimensions. For example, oocytes microinjection, although requires good manual skill and a long training, is facilitated by the size of the cell. Chapter eleven describes a method for detection of

separate activity in mouse oocytes *in vivo*; chapter twelve describes techniques that address the manipulation of meiotic cohesin levels in mouse oocytes describing first how cohesin can be removed from meiotic chromosomes and secondly how the expression can be induced during different stages of oocyte development by using genetically modified mouse strains. The localization of the right protein to the right place in the cell at the right time is the topic of the following chapter entitled “*optogenic manipulation of mouse oocyte*”. The microtubule dynamics using photoactivable GFP-tubulin and the photoactivation of actin in mouse oocytes are the topics of chapters fourteen and fifteen. Photoactivation is very useful technique to study the proteins dynamics and these chapters provide useful information that can be adapted to any protein and during different stages of oocytes maturation and embryo development.

Mechanical forces of the cell are studied using two methods described in the last two chapters of this book: the laser ablation applied to the study of mitotic spindle formation and chromosome segregation (chapter sixteen) and micropipette aspiration to assess cortical tension in mammalian oocytes (chapter seventeen).

I appreciate the editors’ efforts to highlight the more recent topics pertaining to the mouse oocyte development as well as the way the book was structured. Each chapter presents not only detailed and reproducible methods (troubleshooting included) but also nice images and instructive figures providing non-verbal information necessary to better understand how to deal with this “*fantastic little laboratory of molecular biology*” that is the oocyte.

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