Preface

Mammalian oocytes occupy a critical nexus in reproduction as they not only contribute half the genetic makeup of the embryo but also provide virtually all of the cytoplasmic building blocks required for sustaining embryogenesis. The journey that transforms a primordial germ cell into a mature oocyte (or egg) capable of fertilization and embryonic development is of unrivalled complexity characterized by a discontinuous stop–start tempo that spans an astounding period lasting up to four to five decades in humans. Built into this protracted process of oogenesis are two consecutive meiotic nuclear divisions, meiosis I and meiosis II, which proceed without an intervening phase of DNA replication thereby ultimately halving the chromosomal content.

Oogenesis begins in the fetal ovary when the earliest oocyte precursors commit to meiosis and undergo reciprocal recombination. Recombination leads to the reshuffling of genes important for diversity within the species and also for the establishment of a link between homologous chromosome pairs critical for setting up the correct pattern of chromosome segregation during meiosis I. Oocytes then enter their first arrest state at the dictyate stage of prophase I with an intact nucleus (or germinal vesicle as it is known in the oocyte) and surrounded by a somatic cell investiture (or follicle) that acts as a private boudoir for each oocyte within the ovary. This suspended state is maintained until cohorts of prophase I-arrested oocytes are induced to undergo a protracted growth phase lasting ~2 weeks in mice and ~10–12 weeks in humans during which the oocyte builds up its repository of mRNAs and proteins. As a consequence, oocyte volume increases over 100-fold to produce some of the largest cells known—the volume of a fully grown mouse oocyte is ~270 pL, whereas that of a PtK1 somatic cell is ~6 pL—all the while remaining in intimate contact with the surrounding follicle, which is also undergoing development in parallel. Fully grown oocytes located within dominant antral follicles respond to hormonal cues and resume meiosis I marked by breakdown of the GV. Over the course of several hours (6–10 h in mice and 24–36 h in humans), oocytes complete meiosis I and progress immediately to meiosis II only to become arrested for a second time at metaphase II. The end-product of this remarkable developmental process is the "mature" egg, now replete with macromolecules and organelles, and poised to support embryonic development if fertilization should occur to break the metaphase II-arrest state.

Given the importance of the oocyte for embryonic development, it is not surprising that the oocyte holds centre stage in fertility clinics and greatly influences the outcome of assisted reproductive treatments (ARTs). In the current economic and social climate in which child-bearing occurs at increasingly older ages, women in their late 30s and early 40s comprise an ever-expanding proportion of fertility patients. For such patients, the single biggest impediment to successful fertility treatment and pregnancy outcome is poor oocyte quality, a biological phenomenon that at present remains elusive at the molecular level and for which there is no effective therapy apart from the use of "good-quality" eggs provided by donors. Poor oocyte quality is also at the heart of other adverse reproductive sequelae such as chromosomally abnormal pregnancies (e.g., Down's syndrome) and miscarriage both of which most often stem from chromosome segregation errors arising during oocyte

maturation. Understanding the biology of the mammalian oocyte is therefore at the heart of mammalian reproductive performance and a priority agenda for reproductive biologists. Notably, due to its remarkable property for nuclear reprogramming, the mammalian oocyte is also a central focus of stem cell research.

The paucity of human oocytes for research has meant that surrogate mammalian models, most notably the mouse, have been indispensable for elucidating oocyte mechanisms. The aim of "*Mammalian oocyte regulation: Methods and Protocols*" is to provide a highly diverse compendium of detailed methodologies—primarily focusing on the murine model but also including chapters on human oocytes—for enabling researchers to interrogate every aspect of mammalian oocyte development including recombination, meiotic maturation, oocyte substrate uptake, chromosomal segregation, and fertilization.

The chapters cover generic techniques such as those required for obtaining oocytes from female mice (oocytes have to be obtained from hormonally primed live hosts and cannot be propagated in culture in the lab), for stripping oocytes of their surrounding cumulus cells, for oocyte handling and microinjection, and for the in vitro manufacture of cRNAs (important for experimental protein overexpression in the transcriptionally quiescent oocyte). Superimposed on these generic technical descriptions is a diverse range of protocols having more specific focus. These include protocols for studying recombination using allele-specific PCR; for analyzing high-resolution details within the voluminous oocyte using immunofluorescence and electron microscopy; for studying substrate uptake and protein turnover using radioactive reagents, kinase assays, and timelapse fluorescence imaging; for studying chromosomal structure, chromosomal compliment, and chromosome-centred spatial regulation using chromosome spreads, immunofluorescence, FISH, microarray-CGH, FRET technology, and oocyte bisection; for studying germ cell development in relation to the unique ovarian niche using immunohistochemistry of ovarian sections; for studying gene function using long double-stranded RNA for RNA interference and morpholino antisense technology; and for exploring nuclear reprogramming and generating ES cells using nuclear transfer techniques.

This book has strong appeal for the novice oocyte researcher as leading oocyte labs share with readers their unique experience acquired over many years regarding techniques that are core to work in the field; this book therefore represents one of the largest individual collections of "insider secrets" for circumventing unique obstacles within this challenging experimental system. With the wide range of protocols described herein, this book will also be of great interest to experts who wish to add another dimension to their established portfolio. By describing techniques of wide interest such as reporter gene technology, gene silencing, chromosomal analyses, and nuclear transfer, this book will also be a great companion to the wider research community.

Above all, I would like to express my heartfelt gratitude to all the authors who made this book possible by sharing their wealth of expertise in such a selfless, thoughtful, and meticulous manner.

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