Mechanism and Control of Animal Fertilization

Edited by
John F. Hartmann

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Preface

The successful union of a sperm and egg, eventually resulting in the formation of a new individual, is an impressive example among biological phenomena of the operation of sensitive control mechanisms. Indeed, Professor Einstein's famous admonition to the quantum physicists of his day that "God does not throw dice" finds dramatic application in describing the harmonious interactions required of fertilization.

An understanding of the molecular mechanisms involved in animal fertilization has just begun. Studies of invertebrate gamete interactions, particularly those of the sea urchin, have laid much of the groundwork toward acquiring an understanding of these systems. The availability of large numbers of gametes capable of undergoing synchronous fertilization in an inexpensive medium has been a blessing for students of fertilization and early development for more than a century. Those working with mammalian gametes, on the other hand, have had to struggle with less than abundant quantities of cells and artificial *in vitro* systems, the use of which have, nevertheless, begun to yield answers. Of course mammalian fertilization does not occur in the test tube (normally), so a complete understanding of this phenomenon must take into account the influence of the *in vivo* environment, still a formidable consideration.

As is so common in science, methodological difficulties discourage many from entering a given field of study; mammalian fertilization is no exception. However, with the development of systems for increasing the yield of gametes, in particular of the egg, combined with highly sensitive analytical techniques, both of which are discussed in some of

the chapters in this volume, it is hoped that more investigators will be induced to enter this area.

The purpose of this book is to review many of the contributions that the study of both invertebrate and vertebrate systems has made toward elucidating the mechanism of animal fertilization. The majority of the chapters deals with the mammal, which simply reflects the current prejudice of the editor. Informally, the chapters can be grouped into the following sections: (1) formation of gametes (oogenesis and spermiogenesis), (2) composition and response of gamete surfaces (zona pellucida, sperm capacitation, and membrane behavior as reflected by ionic movements in invertebrate egg), (3) prepenetration interactions between sperm and eggs of both invertebrates and mammals, (4) early postfertilization changes (block to polyspermy in the invertebrate, mammalian, and anuran egg; early synthetic and other changes in the fertilized mammalian egg), and, finally, (5) aspects of *in vivo* fertilization in the mammal (interaction of sperm and egg and gamete transport in the female reproductive tract).

John F. Hartmann

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1

Oogenesis: Synthetic Events in the Developing Mammalian Egg

PAUL M. WASSARMAN

I. INTRODUCTION

The unfertilized mammalian egg represents the culmination of oogenesis, a complex developmental process that begins in the fetus and terminates with ovulation by sexually mature offspring. As a result of the process of oogenesis each ovulated egg has the potential to

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give rise to a new individual who will express and maintain the characteristics of the species.

Among the consequences of oogenesis are an increase in genotypic variation due to crossing over and recombination, a decrease in gamete ploidy to the haploid state, and the accumulation of macromolecules and organelles that will be used to regulate and sustain early embryogenesis. It is the latter aspect of mammalian oogenesis that will be emphasized here. In particular, this chapter reviews synthetic events that occur during oocyte growth and during conversion of oocytes into unfertilized eggs in the mouse. This chapter is not intended to be a comprehensive survey of the field or of all of the relevant literature. Rather, it is intended to be more of an introduction to this important aspect of mammalian developmental biology.

II. OOGENESIS IN THE MOUSE: A PRÉCIS

A brief description of oogenesis in the mouse is presented below and is summarized in Fig. 1. The information presented is drawn from a variety of sources to which the reader is referred for a more detailed account of the subject (Parkes, 1956; Zuckerman, 1962; Austin and Short, 1972; Biggers and Schuetz, 1972; Zuckerman and Weir, 1977; Jones, 1978; Van Blerkom and Motta, 1979). The text edited by Jones (1978) is, perhaps, the most up-to-date, comprehensive treatment of mammalian oogenesis available.

A. Appearance of Oocytes during Fetal Development

Oogenesis in the mouse begins with the formation of primordial germ cells in presomite embryos. In the 8-day-old embryo, containing four pairs of somites, about 100 primordial germ cells are recognizable due to their distinctive morphology. These large cells are found in that region of the allantois arising from the primitive streak. Consequently, the embryonic rudiment of the allantois and the caudal end of the primitive streak may be considered the regions of primordial germ cell formation. The primordial germ cells migrate by ameboid movement into the endoderm and then along the dorsal mesentery of the genital ridges found in the roof of the coelom. These primordial germ cells are the sole source of adult germ cells. In the 13-day-old embryo (52–60 pairs of somites), containing a differentiated ovary, migration of primordial germ cells is complete, with virtually all of the cells converted to actively multiplying oogonia ($\simeq$ 95% of the germ cells) or

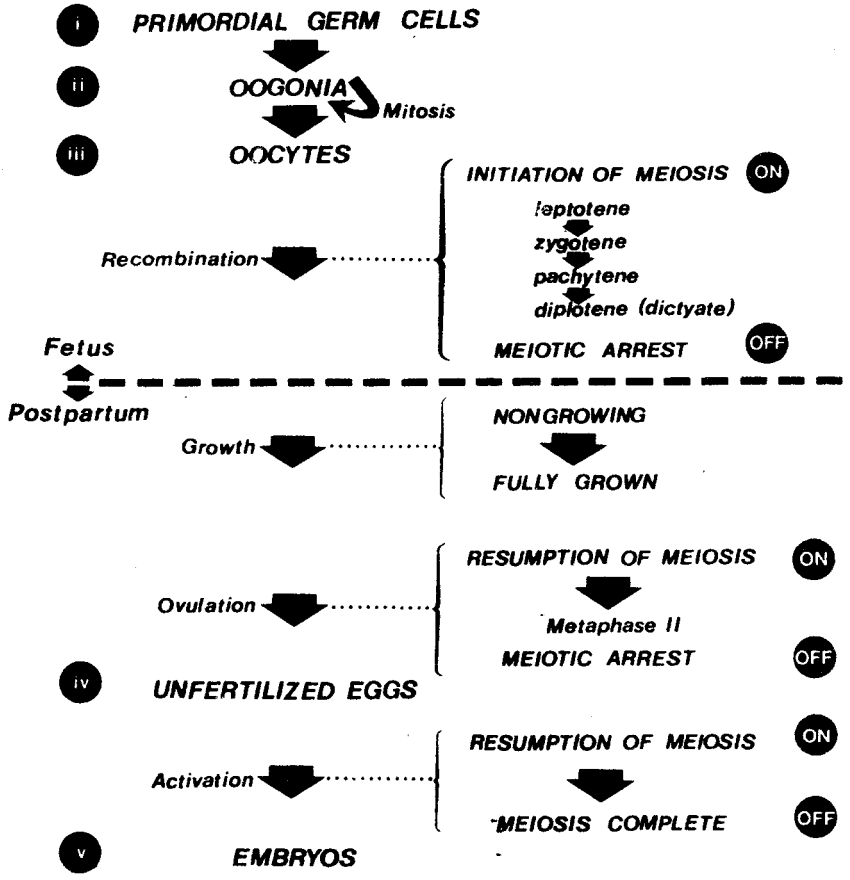

Fig. 1. A summary of the sequence of events that occurs during oogenesis in the mouse. For details see Section II,A–D.

to oocytes (≃ 5% of the germ cells) in leptotene of the first meiotic prophase. By day 14 of embryogenesis (61–62 pairs of somites) the germ cell population is about equally divided between oogonia and oocytes, and by day 17 (full quota of 65 pairs of somites) the ovary contains only oocytes at various stages of the first meiotic prophase.

As early as day 12 of embryogenesis, a few oogonia enter the preleptotene and then leptontene stage of the first meiotic prophase. It is during preleptotene (interphase following the last mitotic division of oogonia) that the final DNA synthesis takes place in preparation for meiosis. This synthetic activity signals the transformation of oogonia into oocytes. Oocytes progress rapidly through leptotene (3–6 hours)

and then take 12–40 hours to complete zygotene. During zygotene homologous chromosomes pair and synapse to form what often appear to be single chromosomes but are actually bivalents composed of four chromatids. By day 16 of embryogenesis nearly all oocytes are in pachytene of the first meiotic prophase; a stage that lasts about 60 hours and involves genetic crossing over and recombination. Therefore, nuclear progression from leptotene through pachytene takes approximately 4 days to complete. The first oocytes in the diplotene stage of the first meiotic prophase are seen by day 18 of embryogenesis, with their chromosomes exhibiting the chiasmata that result from crossing over. By the time of parturition a majority of oocytes have entered the late diplotene, or so-called dictyate stage, and by day 5 postpartum nearly all ooctyes have reached the dictyate stage where they will remain until stimulated to resume meiosis at the time of ovulation.

B. Growth of Oocytes and Follicles

Shortly after birth, the mouse ovary is populated with thousands ($\simeq$ 11,000) of small ($\simeq$ 12 μm in diameter), primary oocytes arrested in late prophase of meiosis and enclosed within several squamous follicular cells. There is a loss of about 50% ($\simeq$ 6000) of these oocytes during the first 2 weeks after birth, attributable in large measure to oocytes leaving the ovary through the surface epithelium. However, in the first 2 weeks after birth more oocytes begin to grow ($\simeq$ 600 oocytes, or $\simeq$ 10% of the total population) than at any other period in the lifetime of the mouse. Commencement of oocyte growth is apparently regulated within the ovary, the number of oocytes entering the growth phase being a function of the size of the pool of nongrowing oocytes. The oocyte and its surrounding follicle grow coordinately, progressing through a series of definable morphological stages. The oocyte completes its growth in the adult mouse before the formation of the follicular antrum; consequently, the vast majority of follicle growth occurs after the oocyte has stopped growing. Growth is continuous, ending either in ovulation of a matured oocyte (unfertilized egg) or degeneration (atresia) of the oocyte and its follicle.

Completion of oocyte growth in the mouse takes approximately 2 weeks, a relatively short period of time in comparison to the months or years required for completion of oocyte growth in many nonmammalian animal species. The oocyte grows from a diameter of about 12 μm (volume of $\simeq$ 0.9 pl) to a terminal diameter of about 85 μm (volume of $\simeq$ 320 pl), not including the zona pellucida. Therefore, during its growth phase, while continually arrested in dictyate of the first meio-

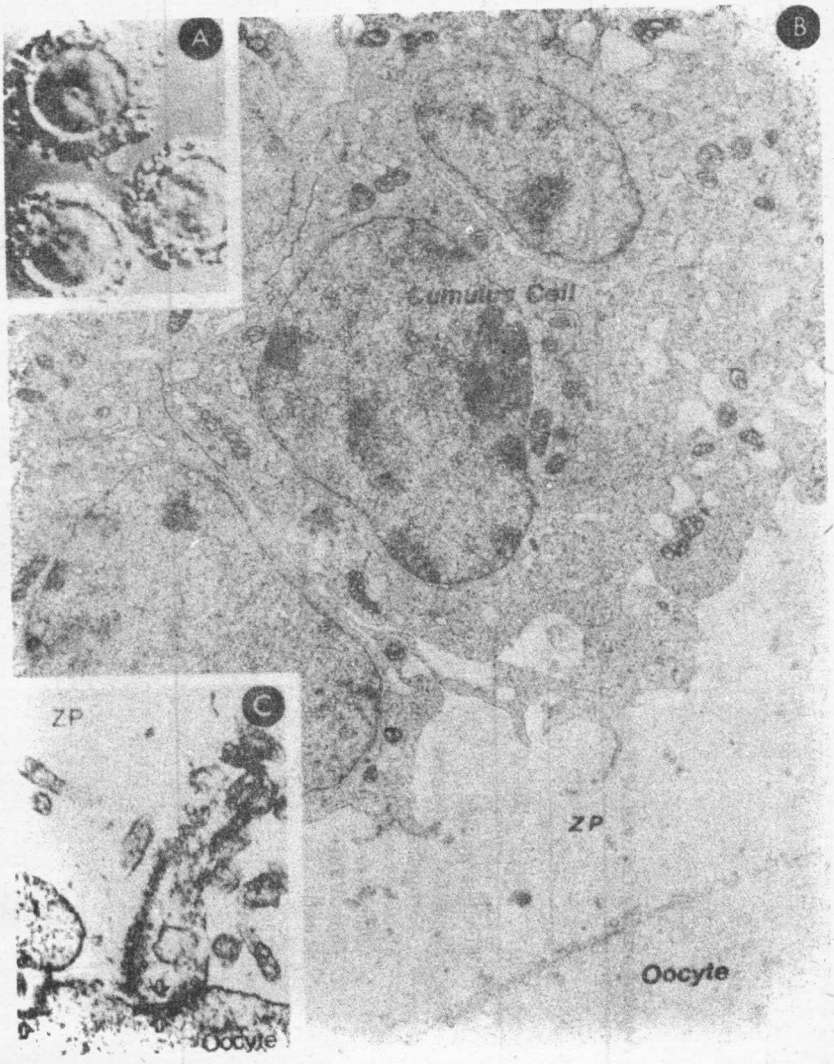

Fig. 2. Light (A) and transmission electron (B,C) micrographs of isolated, fully grown mouse oocytes with their adherent cumulus cells. The arrows in panel C indicate the positions of junctions between plasma membranes of an oocyte and an innermost cumulus cell. zp, Zona pellucida.

TABLE I

Ultrastructural Changes Accompanying Growth of Mouse Oocytes

Organelle or inclusion	Stage of oocyte growth (diameter)		
	Early (≈ 20–40 μm)	Middle (≈ 40–60 μm)	Late (≈ 60–85 μm)
Nucleoli	Fibrillogranular	Larger, fibrillar	Very large, very dense
Mitochondria	Elongated, transverse cristae ("orthodox")	Smaller, round, columnar cristae	Round or oval, concentric cristae ("unorthodox")
Golgi complex	Flattened stacks of parallel lamellae	Parallel lamellae, vacuoles, granules	Swollen lamellae, highly vacuolated, granular
Zona pellucida	Thin, diffuse	Thicker, denser	Very thick, very dense
Endoplasmic reticulum	Smooth	Moderately vesicular	Highly vesicular
Lipid droplets	+	++	++++
Multivesicular bodies	+	++	++++
Cytoplasmic lattices	—	+++	++++++
Cortical granules	—	+	+++