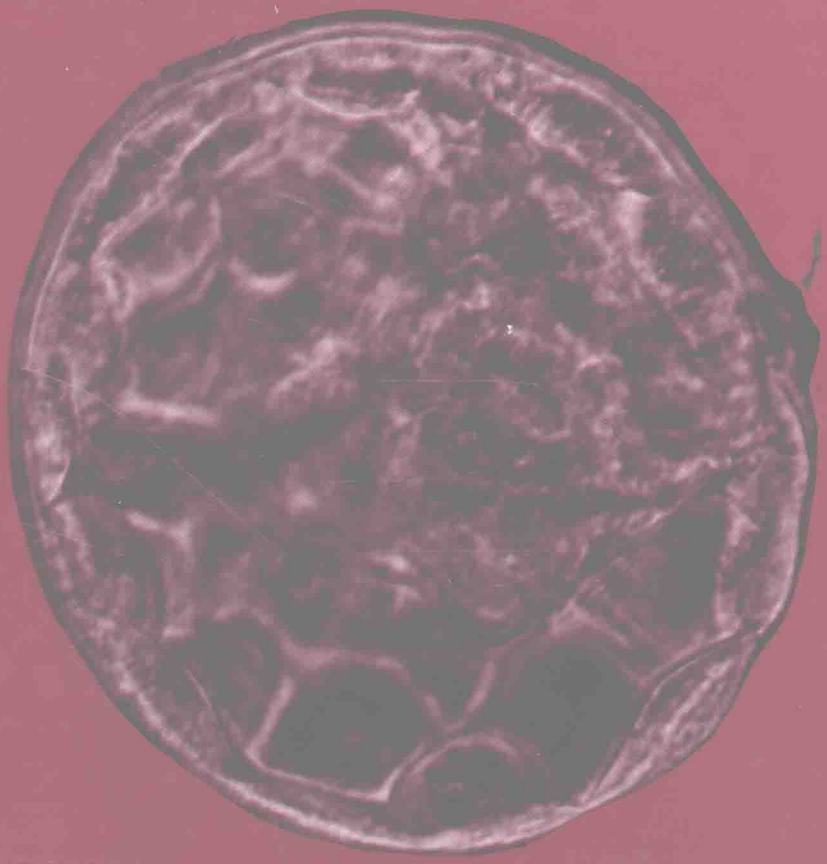

Atlas of the Human Oocyte and Early Conceptus

Lucinda L. Veeck

With a Foreword by
Howard W. Jones, Jr.

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WILLIAMS & WILKINS
Baltimore • London • Los Angeles • Sydney

Editor: Carol-Lynn Brown
Copy Editor: Deborah Tourtlotte
Design: JoAnne Janowiak
Illustration Planning: Wayne Hubbel
Production: Anne G. Seitz

Printed in the United States of America

Library of Congress Cataloging-in-Publication Data

Veeck, Lucinda L.
Atlas of the human oocyte and early conceptus.

1. Fertilization in vitro, Human—Atlases.
2. Oogenesis—Atlases. 3. Ovum—Atlases. I. Title. [DNLM: 1. Fertilization in vitro—atlases. 2. Oocytes—growth & development—atlases. WQ 17 V417a]
RG135.V44 1987 618.1'78'059 86-9211
ISBN 0-683-08589-1

Composed and printed at the
Waverly Press, Inc.

86 87 88 89 90
10 9 8 7 6 5 4 3 2 1

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"OMNE VIVUM EX OVO"
(all life from eggs)

WILLIAM HARVEY
De Generatione Aminalium (1651)

Foreword

During the last 5 years, there has been a far greater opportunity to observe the living human oocyte than in all previous history. It is astonishing to realize that prior to the birth of the first child resulting from the fertilization of an oocyte *in vitro* in 1978, only a handful of scientists had ever seen a living human egg.

This circumstance accounts for the fact that when *in vitro* fertilization became a reality there existed no comprehensive scientific description of the living human oocyte or the dynamic events of human fertilization and early development. All available descriptions were of fixed and sectioned material. Indeed the events of mammalian oocyte maturation, fertilization, and early development were worked out primarily in nonprimate species and were only presumed to occur similarily in the human.

The current status of our knowledge about mammalian egg maturation, fertilization, and early development has been achieved by tedious and tiring steps by a large number of inquisitive investigators in many lands using many species.

Our current understanding is quite recent. It is enlightening and humbling to learn what medical students were taught about these matters a century ago by reading part of a lecture in obstetrics by William Tyler Smith delivered at the Saint Mary's Hospital in London in 1858. Dr. Smith said:

"Gentlemen:

When de Graaf in 1673 described the follicles they were supposed to be the actual ova but in 1827 von Baer in St. Petersburg discovered the true *ovule* within the Graafian follicle. This ovule becomes imbedded in an aggregation named the cumulus. The ovule itself is found to consist of an external membrane, the *zona pellucida*, a yolk and a cell termed the *germinal vesicle* or germ cell.

The germinal vesicle or germ cell was discovered by Purkinje in 1825 in the bird and later in the mammal by Coste.

In 1835 Wagner of Gottingen and Wharton-Jones in England discovered a nucleus in the germinal vesicle which has been called the germinal spot. This addition to our knowledge brought the germinal vesicle within the category of ordinary nucleated cells.

It may now be considered as an established fact that during menstruation Graafian follicles are ruptured and ovules discharged. As the ovule approaches maturity and makes its exit from the Graafian follicle a

remarkable change occurs in the germinal vesicle. It is believed by almost all observers either to disappear or to become entirely transformed, producing two cells in the center of the yolk from which cells the embryo is afterwards developed.

The male element required for conception is the *spermatozoon*. It has been proved by direct experiments such as filtering the semen and using the spermatozoa and the liquor seminis that it is the spermatic particles and not the liquor seminis which are the real agents of fecundation.

In 1843 Martin Barry published an account of the actual penetration of the ova of the rabbit by the spermatozoa. This view was combatted by many, especially Bischoff and Newport, who believed that the spermatozoa approached the ovule, became applied to the surface and disappeared by a process of disintegration. Others have demonstrated the penetration of the ovule by the spermatozoa in the clearest manner. Impregnation does not depend on the penetration of the ovule by one spermatozoon but by considerable numbers of spermatozoa. The greater the number of spermatozoa in apposition to an ovule the more certain its fecundation. It is not by the influence of particle upon particle but of mass upon mass that the phenomena of impregnation are accomplished. After the penetration of the ovule, the spermatozoa lose their motor power, become disintegrated or dissolve and disappear, not, however, until they have communicated to the ovum that wonderful force which leads to the formation of a future being but without which the ovule dies like any other simple animal cell.

The ovule having thus been converted into the ovum by the process of fecundation, we have now to speak of the earliest phenomena observed in development. The first change known to occur is the cleavage or segmentation of the yolk. The embryo cell first elongates, then becomes violin-shaped, and afterwards separates into two cells by spontaneous fusion, an observation first made by Swammerdam. With this division of the embryo cell, the yolk becomes two masses and the two cells are now in the center of the two portions of the yolk. By similar process, the two cells divide into four, then into eight and then form a homogeneous mass called by some authors the mulberry mass."

Thus, we have progressed during the last 100 years and now know that it is the structure described by Smith as the "ovule" which is really the germinal cell. This lecture makes us understand why the germ cell nucleus is still referred to as the germinal vesicle. What Wagner and Wharton-Jones thought was the nucleus of the germ cell and called the germinal spot, we now know as a large nucleolus.

Our forefathers did recognize germinal vesicle breakdown as a "remarkable change" associated with ovulation but thought that this seminal event accompanied menstruation. Imagine having to filter the semen to determine if the spermatozoa or the liquor seminis was the real agent of fecundation, and imagine believing that it was the action of "mass upon mass" which enabled the spermatozoa to deliver "that wonderful force" which resulted in a future being.

The compound light microscope and, of course, the electron microscope have been powerful agents in unraveling these basic biological phenomena by facilitating

the examination of every step of the way in fixed specimens. Now, when observing the living cell in the laboratory, the investigator can deduce, at least in part, the continuously moving, complex events of oocyte maturation, fertilization, and development, understanding of which is so necessary for those who wish to accomplish these events *in vitro.*

Lucinda Veeck has provided us with the missing compendium of the various stages of the maturation of the living human oocyte, its fertilization, and subsequent development as seen by the compound light microscope. These photographs and their descriptions are not only of intrinsic interest but also of surpassing help to those in the laboratory who on a day-to-day basis must evaluate and handle the eggs and fertilize them in programs of *in vitro* fertilization.

Howard W. Jones, Jr., M.D.
President
Jones Institute for Reproductive Medicine

Preface

Aristotle, who walked this earth some 300 years before Christ, said, "He who sees things grow from the beginning will have the finest view of them." It is from this respectful viewpoint, and with at least some sense of awe, that we in *in vitro* fertilization laboratories generally approach our work with human oocyte and conceptus culture. One cannot resist being charmed by the perfection in form of the mature, living oocyte or being fascinated by the movement and dance of a healthy sperm. By observing embryo development which leads to the birth of a human infant, we become aware of our responsibility to nurture and protect these cells in the environment of our clinical laboratories, and we realize that we must educate ourselves in the process, in order that we might improve our technical abilities to handle them.

In March 1980 the clinical application of human *in vitro* fertilization (IVF) was first applied in Norfolk, Virginia, to infertile couples seeking treatment after conventional infertility therapy had proven unsuccessful. During the 5½ years since those first clinical trials, more than 8000 human oocytes have been harvested from gonadotropin-stimulated follicles. These oocytes have represented various stages of maturation, viability, and integrity. Many have been mature (metaphase II; preovulatory) and potentially fertilizable, some less than mature (metaphase I; prophase I; unripened) but capable of *in vitro* maturation, and some have been obviously nonviable (degenerative; atretic) or have represented pathological conditions. Later developmental capability after fertilization and culture *in vitro* has varied as well. One constant aspect of laboratory protocols has been to photograph oocytes and concepti at intervals during the 2- to 3-day course of the collection, fertilization, and growth processes. This practice has contributed to the collection of many thousands of photographs, not only valuable for education and reference but also integral as part of each individual patient's history and record.

Students and visitors to the Norfolk IVF laboratory have been offered the opportunity to study these photographs in our effort to share acquired knowledge and experiences. It has always been our belief in this program that all aspects of this particular science/art should be freely shared. As a result, many requests have followed for the publication of photographs in an atlas format. If such a publication proves of interest or assistance to established IVF centers, newly formed IVF programs, or students of reproductive medicine or embryology, we will feel gratified in the effort.

Most of the material selected for this publication was photographed with the use of a Nikon F3 camera body attached to a Nikon Diaphot inverted microscope (Nikon, Inc., Garden City, NY). With the exception of an occasional phase contrast plate, nearly all photomicrographs were taken with brightfield optics, the author's preference for oocyte and conceptus evaluation. Differential interference contrast accessories were not utilized because of the light defraction difficulties encountered with the use of plastic culturing vessels. Oocytes and concepti were photographed through the plastic surfaces of welled organ culture dishes (#3037, Falcon Plastics, Oxnard, CA) containing approximately 3 ml of culture medium. Occasionally, oocytes involved with dense and obscuring cumulus cell formation were photographed in lesser medium volumes in flat bottom Petri dishes (#3002, Falcon Plastics). It should be stated that since all material presented here was collected during the course of an ongoing clinical IVF program, more attention was given to rapid and careful handling of specimens than was given to the perfection of photographic technique.

It is sincerely hoped that this book, through the use of pictorial examples, descriptions, and follow-up information, will familiarize the reader with the human female gamete and early conceptus.

The author wishes to thank Nikon, Inc. for the long-term use of the Diaphot microscope, camera, and accessories used for almost all evaluation and photography in this book. Appreciation is also extended to the clinical and surgical teams responsible for providing material for the IVF laboratory, most notably Drs. Howard W. Jones, Jr., Georgeanna S. Jones, Jairo E. Garcia, Anibal A. Acosta, Zev Rosenwaks, and Suheil Muasher, as well as the operating room nurses, Margaret Whitfield and Deborah Perry. In addition, it should be recognized that the unfailing support of co-workers and technicians in the laboratory has enabled the proper collection and identification of photographic material. In this regard, sincere appreciation is given to Jeanne McDowell, Mary Maloney, Simonetta Simonetti, Bruce Sandow, and Jacob Mayer. Artists Patrice Raum and Susan Grupp are responsible for all line drawings and sketches. Andy Andrews and Isaiah Robinson of the Norfolk General Hospital Audiovisual Resources Center provided the cheerful and prompt service of hundreds of hours of film processing and printing. The author also wishes to thank Dr. Maria Bustillo for photographs of *in vivo* fertilized concepti, Dr. R. G. Edwards for the use of morula and blastocyst photographs, Dr. Charlotte Schrader for editorial assistance, and Alan Veeck, my son, for assistance in data compilation and manuscript preparation.

Lucinda L. Veeck
December 1985

Contents

A Review of Meiosis in the Female

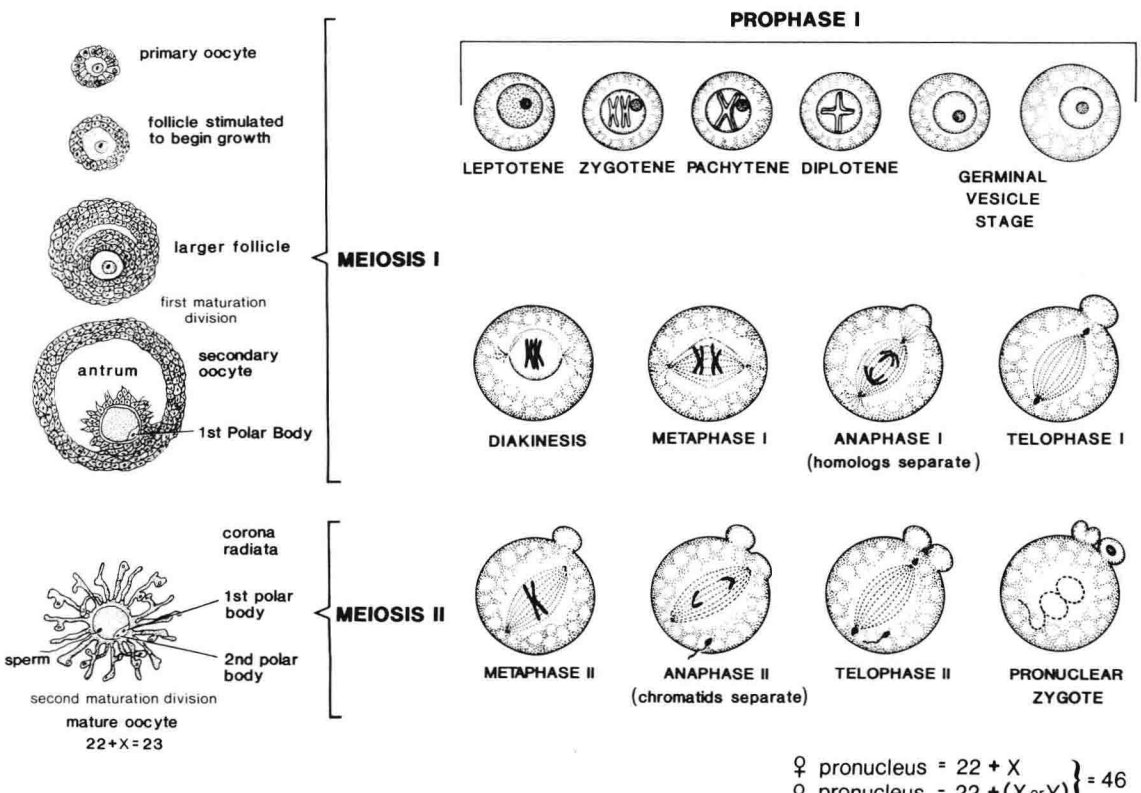
Once ovarian differentiation has taken place, female germ cells are called "oogonia." Only a few hundred oogonia are present in the human ovary near the end of the first month of fetal gestation, but these cells divide mitotically over a span of several months before the process of meiosis is initiated. The enormous increase in the number of oogonia produced by these mitotic divisions results in each female starting her fertile life with somewhere around half a million germ cells. Shortly after the last mitotic division of the oogonium, the cell enters an interphase during which time DNA is replicated in preparation for meiosis (preleptotene stage). In the human ovary the transformation of oogonia to oocytes by entrance into meiotic prophase begins from the third month of gestation. By the time of birth all germ cells are oocytes, and the meiotic process is arrested (first meiotic arrest). The oocyte, with a prominent nucleus (germinal vesicle), rests in the dictyate or dictyotene stage, which persists through infancy until just prior to ovulation.

Meiosis is termed "cell reduction" because it reduces the number of chromosomes in each daughter cell to the haploid (n, 23 chromosomes) number. When a mature oocyte with a haploid number of chromosomes unites with a mature spermatozoon of haploid number, a diploid (2n, 46 chromosomes) zygote is formed. Meiosis differs from mitosis in its reduction of the 4n (postsynthetic) nucleus to four "n" or haploid nuclei. Meiosis also creates new gene combinations in the chromosomes by mixing parent chromosomes. For example, each oocyte contains a pair of number three chromosomes, one from each of its parents. During meiosis I, these two homologs exchange genetic material between them so that oocytes inherit chromosomes that are derived from, but not identical with, their parents' homologous pair. This genetic exchange occurs between each chromosome pair and is responsible for the variation of phenotype found in all sexually reproducing organisms. It may also be responsible for the selective advantage that some oocytes display over others in regard to cell viability.

Meiosis has two stages, MI and MII. During MI the homologs separate; during MII the chromatids separate. The result of this is four cells (theoretically), each with a haploid set of chromosomes. This model may not hold strictly true for all oocytes because first polar bodies may not participate in second divisions. Unlike

meiosis in the male gamete, female meiosis results in only one viable oocyte with the bulk of ooplasm, the other cells becoming polar bodies. Both stages of meiosis have a prophase, metaphase, anaphase, and telophase. Prophase I is complex and is divided into five substages: leptotene, zygotene, pachytene, diplotene, and diakinesis.

I. MEIOSIS I
   A. *Prophase I*
      1. *Leptotene:* Nuclear chromatin begins to condense; both ends of the chromosomes are attached to the nuclear envelope. Under the microscope the oocyte has an enlarged nucleus.
      2. *Zygotene:* Homologous chromosomes align themselves side by side and attach to each other, thus allowing homologous loci to come into contact ("synapsis"). A definite structural material, termed the "synaptinemal complex," holds the chromosomes close to each other. The exchange of genetic material most likely takes place in these complexes. This exchange of genetic material is called "crossing over" or "recombination." Each set of homologs in synapse is called a "bivalent," since the two chromosomes do not yet appear differentiated into chromatids. Under the microscope the nucleolus is still visible. Zygotene ends when all homologs have been paired.
      3. *Pachytene:* The bivalents shorten and become thick. "Crossing over" (the exchange of linked genes between homologous chromosomes)



occurs at this time. In this stage the bivalent, because of its closely opposed chromatids, is termed a "tetrad."

4. *Diplotene:* Bivalent chromosomes come apart except at certain points called "chiasmata." Generally, one or more chiasmata join homologs; these areas are sites of genetic crossing over. Chromosomes take on a cross-like appearance. At this point in females, meiosis is halted before diakinesis. In the female ovary, many thousands of oocytes remain in this stage, termed "dictyotene," until just prior to ovulation or degeneration, perhaps as long as 45–50 years. This stage is marked by the morphological appearance of an intact germinal vesicle.
5. *Diakinesis:* The oocyte begins growth in the antral follicle after puberty under stimulation by unknown factors. Meiosis I is resumed just prior to the time that the oocyte is ready to be ovulated. The nucleolus dissipates and the nuclear envelope disappears. The chiasmata appear to move toward the ends of the bivalents (to "terminalize").

B. *Metaphase I:* The spindle is formed and the bivalents line up at the equatorial plane. In females the spindle is off-center in the cell and by its position determines which of the daughter cells will inherit most of the cytoplasm. Those chromosomes of both maternal and paternal origin are lined up randomly toward the poles, and the whole chromosomes sort independently to either oocyte or first polar body. In this manner, maternal and paternal chromosomes are mixed and can theoretically result in as many as 8 million chromsomally different gametes (2^{23}). This stage is marked by the absence of a nucleus (germinal vesicle) and exists prior to first polar body extrusion.

C. *Anaphase I:* Whole chromosomes, with centromeres intact, move to opposite ends of the cells.

D. *Telophase I:* Cell division occurs, with the main oocyte receiving the bulk of cytoplasm and the first polar body (mostly nucleus) receiving a much smaller portion. The nuclear envelope reforms and the chromosomes remain attached to it until meiosis II. The oocyte and first polar body are connected by a cytoplasmic bridge.

II. MEIOSIS II

A. *Prophase II:* There is no true prophase II in human oocytes because the cell passes directly into metaphase II without replication of DNA.

B. *Metaphase II:* The nuclear envelope again disappears and the spindle reforms. The 23 chromosomes, each with two chromatids, line up again as the equatorial plate. Although there is only a haploid set of double-stranded chromosomes present, there is still a diploid amount of DNA because the strands have not yet separated. This is considered the "ovulatory stage." Ovulated oocytes are arrested at this stage until sperm penetration activates the resumption of meiosis II.

C. *Anaphase II:* Chromatids split at the centromeres and move to opposite ends of the cell.

D. *Telophase II:* Again, the oocyte proper (ovum) retains the bulk of the cytoplasm and the second polar body inherits a smaller amount, mostly nucleus. The first polar body may also undergo meiosis II. Theoretically, the oocyte proper, with the bulk of cytoplasm, is the only viable gamete.

CHAPTER 1

Human Oocytes at the Time of Follicular Harvest

In vitro fertilization laboratories must deal with continual and oftentimes problematic decisions associated with the assessment of oocyte morphology. Because no reliable biochemical test for follicular fluid components has yet been developed to provide accurate and rapid information regarding the maturational status of harvested oocytes, the direct microscopic evaluation of oocyte morphology remains the single most critical parameter on which to base further procedural decisions. In this regard, the following five general points of assessment are taken into consideration for each specimen:

1. *Nuclear status of the oocyte.* While undoubtedly the most important parameter in gamete evaluation, the direct visualization of true nuclear status is often difficult because of obscuring cumulus cell material. Several methods may be used either to clear away or totally remove these cells in an effort to improve visualization, but any method that may interfere with pH stability or create extra environmental stresses on the oocyte should be used cautiously. Oocytes may be stripped of cells with chemical agents (usually hyaluronidase), placed into microdroplets, or put under coverslips to improve visualization. Because of potential dangers with the utilization of such methods, most clinical laboratories do not resort to such techniques unless absolutely necessary. A well visualized oocyte will display (*a*) an extruded first polar body (metaphase II chromosomes); (*b*) no first polar body, no germinal vesicle (metaphase I chromosomes); or (*c*) an intact germinal vesicle (prophase I chromosomes).
2. *Ooplasmic characteristics of the oocyte.* The color, shape, and granularity of an oocyte provide clues regarding its maturity and viability. Ooplasm may appear (*a*) symmetrical or irregular (approximately 100 μm to 150 μm in diameter with zona pellucida); (*b*) homogenous in color or centrally darkened; or (*c*) smooth or granular in appearance.
3. *Appearance of cells most peripheral to the oocyte (coronal cells).* All cellular material surrounding an oocyte is defined as "cumulus," with the innermost and densest layer of cells defined as the "corona." The coronal layer of cells may take on several different patterns in its appearance: (*a*) a "radiant" appearance, similar to a "sunburst" pattern and very expanded; (*b*) a loose, "halo" appearance with slight expansion of these cells; (*c*) a "compact" appearance