Atlas of Fine Structure of Human Sperm Penetration, Eggs and Embryos Cultured *In Vitro*

A. Henry Sathananthan, Ph.D.
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Foreword

This atlas is a fascinating and original visual demonstration at high magnification of the main steps in human reproduction and embryology. It is a most opportune time for its appearance, with the current public and professional awareness of the philosophical, ethical, as well as biological issues arising from the *in vitro* fertilization (IVF) and embryo transfer techniques pioneered by the authors. We are led through a simple, easily understood sequence from development of the egg, sperm penetration, culminating in fertilization followed by embryonic development and formation of organelles. In addition to the normal processes, electromicrographs are included illustrating abnormalities in each of the phases of the reproduction process. The micrographs are of a high standard and the advantage in combining the expertise of the gynecologist, reproductive scientist and embryologist are amply demonstrated by the text. The atlas will be an invaluable aid to the layman as well as to practicing clinicians for understanding the mechanisms and issues involved in human reproduction.

—Professor G. Burnstock, Ph.D., DSc, FAA
Chairman and Head, Department of Anatomy
and Embryology, University College,
London, England, U.K.

Preface

Electron microscopy, an essential technique in both basic and clinical research, has improved our understanding of human reproduction and embryology and provided much of the momentum in the advance of human *in vitro* fertilization (IVF) techniques initiated by Edwards and coworkers in 1969.

The pioneering electron microscopy of Zamboni (1971, 1972) and others (Zamboni et al., 1972) on human oocyte maturation and fertilization *in vitro* and later the original studies by Soupart and Strong (1974, 1975) confirmed for the first time that human spermatozoa could penetrate and fertilize human oocytes *in vitro.* Some idea of the time taken for spermatozoa to penetrate the oocyte investments and form pronuclei, obtained by McMaster, Lopata, Sathananthan, and collaborators (1978, 1980) in our laboratory, and the continuing work on the process of sperm penetration, oocyte maturation, activation, and early embryonic development clearly described by Sathananthan and colleagues (1980–1985) established the credentials of IVF within the scientific community. These studies were also the catalyst for many other scientific studies of early human embryogenesis. For example, the observations of Sathananthan and Trounson (1982a,b) on cortical immaturity of follicular oocytes led to the experiments on delayed insemination (Trounson et al., 1982), which became an established procedure in human IVF, improving fertilization rates and removing time restrictions for the insemination of oocytes. The work reported by Mohr and Trounson (1982) showed that the human embryo can develop in culture apparently normally to the hatched blastocyst stage, and now many studies have described the normal and abnormal ultrastructural morphology of preimplantation human development *in vitro,* which has added significantly to the scant observation of development *in vivo* (Sathananthan et al., 1982b; Sathananthan, 1984; Trounson and Sathananthan, 1984). It is the knowledge derived from all these studies that provided the basis for improvement of the procedures and simplification of techniques used for IVF and for rationalization of advances in IVF and related technologies. The wealth of information derived from innumerable investigations on animal reproduction in the past, embracing electron microscopy, biochemistry, and physiology, has also contributed in no less measure to the overall success of IVF in the human. The electron

microscopic contributions of Bedford (1982) and Yanagimachi and coworkers (1981) on sperm penetration, sperm-oocyte interaction, and cross-fertilization were particularly relevant.

Ultrastructural studies will remain an integral part of new reproductive technology because there is no other technique available at present that can provide anywhere near the same quality of scientific detail on human gametes and early embryos. Clear visualization of events leading to nuclear, cortical, and cytoplasmic maturation, sperm acrosome reaction, sperm penetration and incorporation and subtle abnormalities thereof would not have been possible without electron microscopy. It is the quality of this kind of information that finally determines the accuracy of scientific decision making in research.

Acknowledgments

The authors wish to express their thanks to the following who have helped them in different ways to compile this atlas: the members of the *in vitro* fertilization team of the Queen Victoria Medical Centre for their clinical, laboratory, and research expertise, including Profs. John Leeton and W.A.W. Walters; Drs. P.M. Renou, G. Kovacs, A. Lopata, G. Baker, M. Mahadevan, L. Mohr, and P.A.W. Rogers; and Ms. J. Wood, J. Lutjen, C. Caro, D. Jessup, L. Freeman, R. McMaster, and W. Bishop.

We are indebted to Dr. Peter Campbell, the late Mr. Gary Peace, and Mr. Craig Jackson for the use of the electron microscope and facilities at the Royal Children's Hospital, Melbourne; to Dr. R. Lennie and Mr. Jeff Roles of the Royal Melbourne Institute of Technology for facilities provided for the use of the ultramicrotome; to Ms. F. Collier for printing the micrographs; and to Mr. Dennis Philipatos for technical assistance in processing some of the material.

We extend our thanks to Vera, Johann, Leo, and Theodore Sathananthan who assisted us in putting this atlas together and to Mrs. Audrey Sungaila who typed the manuscripts, for her continued patience and understanding. We are also grateful to Mrs. P. Fry for providing some of the research facilities in the Department of Biological Sciences at the Lincoln Institute.

This research was funded by the Infertility Medical Centre, Melbourne, the National Health and Medical Research Council of Australia, and the Lincoln Institute of Health Sciences, Melbourne.

General Introduction

The only detailed atlas published so far on the fine structure of mammalian oocyte maturation and fertilization is that by Zamboni (1971). This covers predominantly mouse eggs and has few illustrations of human maturation and fertilization. It, however, includes the differentiation and maturation of the human sperm and egg within the testis and ovary and, no doubt, was an excellent contribution at the time. Suzuki's (1973) atlas of mammalian ova has elegant pictures photographed under phase-contrast microscopy, but includes few electron micrographs.

With the advent of *in vitro* fertilization (IVF) as an accepted clinical procedure, it became increasingly necessary that an atlas on the ultrastructure of human sperm penetration, oocyte maturation, fertilization, and early embryogenesis be published to serve the needs of clinicians, scientists, researchers, technicians, and others in the field of IVF and human reproduction. This atlas will also be useful academically to embryologists and cell and reproductive biologists and may be used as a teaching aid for medical and science students in advanced colleges, universities, and research institutes. Improved methods of fixation and processing routinely used today have been employed in this study to make interpretation of ultrastructure and comparisons with other cells easier than before (See Appendix A).

Fawcett's (1981) atlas on the cell and Ghadially's (1982) atlas on its pathology have been sources of much inspiration for the publication of our atlas. After all, the human egg is a basic, undifferentiated cell that will eventually give rise to all the types of cells in the human body, once it is fertilized. It, however, shows certain specialized features that help it fulfill its role in procreation and survival both in and out of the female body. The sperm, on the other hand, is a highly specialized, motile cell, its sole function being penetration of the egg and deposition of paternal chromosomes.

The ultrastructural organization of the human egg closely resembles that of most mammals' eggs. Many of the cellular organelles resemble those found in other mammalian eggs with a few exceptions. The major events relating to maturation, fertilization, and activation are strikingly similar to the general pattern of mammalian development.

The references cited in this atlas are limited mainly to relevant research done on the human. References relating to animal work will be re-

stricted to the many excellent reviews and texts now available in appropriate fields of reproductive biology.

The atlas has been compiled after 8 years of intensive research on the ultrastructural aspects of human IVF, where over 400 preovulatory eggs, both normal and abnormal, have been assessed for maturation, fertilization, and early development. These electron microscopic studies combined with noninvasive methods such as phase and interference microscopy have provided the basis for the improvement and simplification of IVF techniques, which have consequently led to our expertise and achievements in this field of research.

An atlas must be a visual guide, concise, informative, and easily referable. One hundred thirty-six of our own representative micrographs covering various aspects of human IVF have been compiled and organized into 100 figures, in an effort to make this publication as comprehensive as possible. There are, of course, some gaps in our knowledge pertaining to oocyte maturation, sperm/oocyte interaction, and postfertilization events, which need further investigation. Each micrograph is labeled and has been complemented with a detailed legend on fine structure, which may include some introductory information, references to relevant work, and possible clinical applications. Each section has also been provided with a short introduction. Schematic explanatory diagrams show the structural organization of the human sperm, oocyte, and fertilized ovum, where necessary. These are included as five text-figures. A few important light micrographs were added in Appendix B to help the reader visualize the whole egg and relate it to ultrastructure. Some notes that might be useful in the morphological assessment of oocyte maturation, fertilization, and embryo development for IVF and embryo transfer are also included in Appendix C.

An embryologist (AHS), a reproductive scientist (AOT), and a gynecologist (CW) have jointly collaborated in the production of this atlas. The authors have many recent publications in IVF research to their credit. Prof. Geoffrey Burnstock of London University has very kindly written the foreword to this publication.

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Section 1

Introduction to Oocyte Maturation

Maturation of oocytes in the present context includes both nuclear and cytoplasmic events that occur within the oocyte and also cumulus-oocyte interactions at its surface (Thibault, 1977; Soupart, 1980; Szollosi and Gerard, 1983; Crosby and Moor, 1984). Events in these two cellular compartments (oocyte and cumulus) must be fully satisfied before a fertilizable, preovulatory oocyte is produced.

Meiosis consists essentially of two cell divisions. The first involves halving of the chromosome number (diploid to haploid), whereas the second resembles mitosis, although only haploid chromosomes are involved. Homologous chromosomes (one member derived from each parent at fertilization) separate in the first division, and chromatids (arising by the splitting of chromosomes) separate during the second division. Unequal cleavage of the ooplasm at both divisions ensures the formation of polar bodies, which carry the excess chromosomal material.

Meiosis is a long, drawn-out process in female germ cells and is initiated in the fetal ovary, when the process is first arrested at diplotene of prophase (dictyate stage). This oocyte has a prominent nucleus with decondensed chromatin, called the "germinal vesicle," which persists through puberty until it is recruited to complete maturation prior to ovulation. The final stage of maturation begins with germinal vesicle breakdown (GVBD) after the first meiotic arrest and ends when the sperm fuses with the oocyte at fertilization. A second meiotic arrest at metaphase II is interpolated between these two events, when the mature oocyte is released at ovulation. Both follicular and oocyte maturation are involved simultaneously in this process. All of these preovulatory events are