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Title Page

Michael J. Tucker wishes to recognize his friends and family whose love and support helped make this new edition happen, especially the indefatigable Juergen Liebermann, and of course my wonderful wife Megan and children Harry, Nick, Isabella, and Eliza, who had to suffer through all my complaints!

Juergen Liebermann gives most sincere thanks to his wife Maike and his sons and daughter Richard, Lennart Martin, Tobias Georg, Alexander Johannes, and Franziska Sophie for providing unfailing support and encouragement to bring this work to reality.

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Foreword

In vitro fertilization (IVF) is, in the majority of cases, the most direct and effective solution to the current epidemic of infertility surreptitiously plaguing modern societies and wreaking havoc with their economies, while causing the grief of unwanted childlessness to ruin people's lives. Of course, IVF also poses a host of problems, but many of these can now be resolved with vitrification.

For example, most women will have more than one embryo, and before clinical use of vitrification, slow freezing of surplus embryos was problematic, yielding only inconsistent survival and pregnancy rates. Consequently, there was a strong temptation to transfer more than one or two fresh embryos to increase chances of pregnancy—thus elevating the risk of a high order of multiple pregnancies, which, as an unintended consequence, has been IVF's biggest problem. However, with the more recent introduction of vitrification technology, we are able to cryopreserve extra embryos that are not transferred, and to assure patients that this process will neither damage their embryos nor necessarily lower their ultimate cumulative pregnancy rate. Indeed, clinical experience is now showing that pregnancy rates from "frozen/thawed" embryos may be even higher than those from "fresh" embryos.

The benefits of vitrification also extend beyond embryo cryopreservation, being applicable to both oocytes and ovarian tissue freezing, and including even the whole ovary. "Egg freezing" had always been somewhat of an unfulfilled dream, whereas, at least with embryo slow freezing (the norm for two decades), clinical outcomes following thawing yielded adequate survival much of the time, but still gave a distinctly lower pregnancy rate than did transfer of fresh embryos. Slow freezing of oocytes was never able to provide clinically reliable outcomes, and remained largely sidelined as a routine procedure. The reasons are severalfold: (1) the mature oocyte is the biggest cell in the body and contains a high proportion of water, making it difficult to remove enough water during slow freezing to avoid ice crystal formation; (2) the mature oocyte, with chromosomes precariously aligned on the metaphase II spindle, is extremely sensitive to damage from ice crystal formation and may easily be disrupted; (3) the oocyte is also exquisitely vulnerable to mild chilling, which may cause significant compromise or even complete degeneration. So application of an "ultra-rapid freeze" approach with vitrification allows a consistent solution to all these problems, and has ushered in a new era of successful "egg freezing." This has kickstarted the "donor egg bank" industry, has enabled easy use of vitrification to cryostore eggs if a husband is unable to provide sperm on the day of an IVF egg retrieval, and, perhaps most importantly and of greatest media interest, has addressed the "ticking biological clock" haunting the reproductive potential of women.

The major cause of the current infertility epidemic is that women are putting off childbearing until they are older, because of better opportunities for career and education. Many women today do not consider having a baby until their mid-thirties or even later, and, by then, over 20% are infertile; by age 40, the vast majority are infertile simply because their eggs have aged significantly. However, cancer patients who have had their eggs or ovary frozen before undergoing sterilizing chemotherapy and radiation do not have to worry about this widespread concern, because they have young eggs or ovary safely in the freezer. With vitrification, we can now achieve, in all women, that dream of safely preserving fertility in youth to protect them from the biological clock's relentless progress.

Innovating vitrification in the 1980s, and then perfecting it so that it would be more reliable in the early 2000s, was not an easy journey for its clinical pioneers, such as Rall and Fahy in the mid-1980s in the United States, and Kuwayama in Argentina and Japan in the 1990s, and up to the present. IVF clinicians were reluctant to leave the comfortable space of old ideas and the use of "slow freezing," and often ridiculed vitrification; after all, it seemed too easy, when in fact it was very difficult, simply because it has to be just exactly right to achieve success. It took much experimentation with many protocols; and having found the right one, it was most important not to tamper with it and not to deviate from the details. Cryobiology can be a very empirical science, with much painstaking trial and error (of course, based on scientifically derived postulates) over many years until you finally have the knowledge to get it right. It is not an easy procedure, with numerous points in the vitrification process of just one oocyte, and if a step is inadvertently not carried out perfectly, it spells disaster for that oocyte.

The purpose of this book, therefore, is to provide readers with all the information they will need to perfect vitrification in their own laboratories. Dr. Michael Tucker and Dr. Juergen Liebermann include every major scientist who has worked on vitrification in recent times, and describe how these individuals brought us closer to this clinical breakthrough. In addition, readers will understand the controversies and pitfalls of the process, and how to resolve and avoid such issues, allowing them to carry out a perfect job in their own IVF lab and make the dream of preserving fertility a clinical reality.

Dr. Sherman Silber

Preface

Assisted reproductive technology (ART) has been evolving for more than 30 years, and cryopreservation has become one of the keystones in such clinical infertility treatment. However, the true "explosion" in many embryologists' minds in recognizing the true value of cryopreservation occurred with the appearance of vitrification techniques in the mid-1980s. Very gradually since that time, vitrification has established itself as "the cryopreservation technique of choice," through consistency and predictability in terms of the quality of the cell survival following the vitrified "state of suspended animation." Using a vitrification approach, oocyte freezing has become a reality and routine, not to mention embryo vitrification, which in turn provides outcomes comparable to those achieved with fresh transferred embryos. Furthermore, vitrification has opened the doors for new areas in the field of ART such as oocyte banking, preimplantion diagnostics at the blastocyst stage, and notably it has helped to fuel the burgeoning adoption of elective single-embryo transfers. This final area of impact is probably the most important development, because it defines "quality of ART" in a new light—the delivery of a healthy single baby in each IVF cycle.

The success of cryopreservation in benefiting the very specialized field of ART has arisen only after much effort and is nothing short of a small miracle, given that it relies on such an intensive cooperation between science and clinical application. The development of gamete and embryo vitrification specifically represents the triumphant culmination of several decades of frustrating exploration with "slow freeze" technology. The first edition of this book, published in 2007, was subtitled: "A user manual and trouble-shooting guide." This second edition, being published more than 7 years later, has evolved into a more comprehensive book now containing chapters reporting the successful application of vitrification from research up to the level of its use in routine clinical therapy. We as editors of this book are excited, pleased, and grateful to have chapters contributed by scientists and clinical embryologists who have been involved in the development of vitrification at the very highest level. The list of contributors demonstrates a global collaboration of professionals who have given their time, energy, and intellect to share their scientific observations and clinical experiences with the future readership of this book and beyond.

Our destiny working in the field of IVF every single day is based on strong values, and one of these is to fulfill the dreams of our patients in creating healthy families for them; vitrification plays an increasingly essential role in achieving this goal.

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