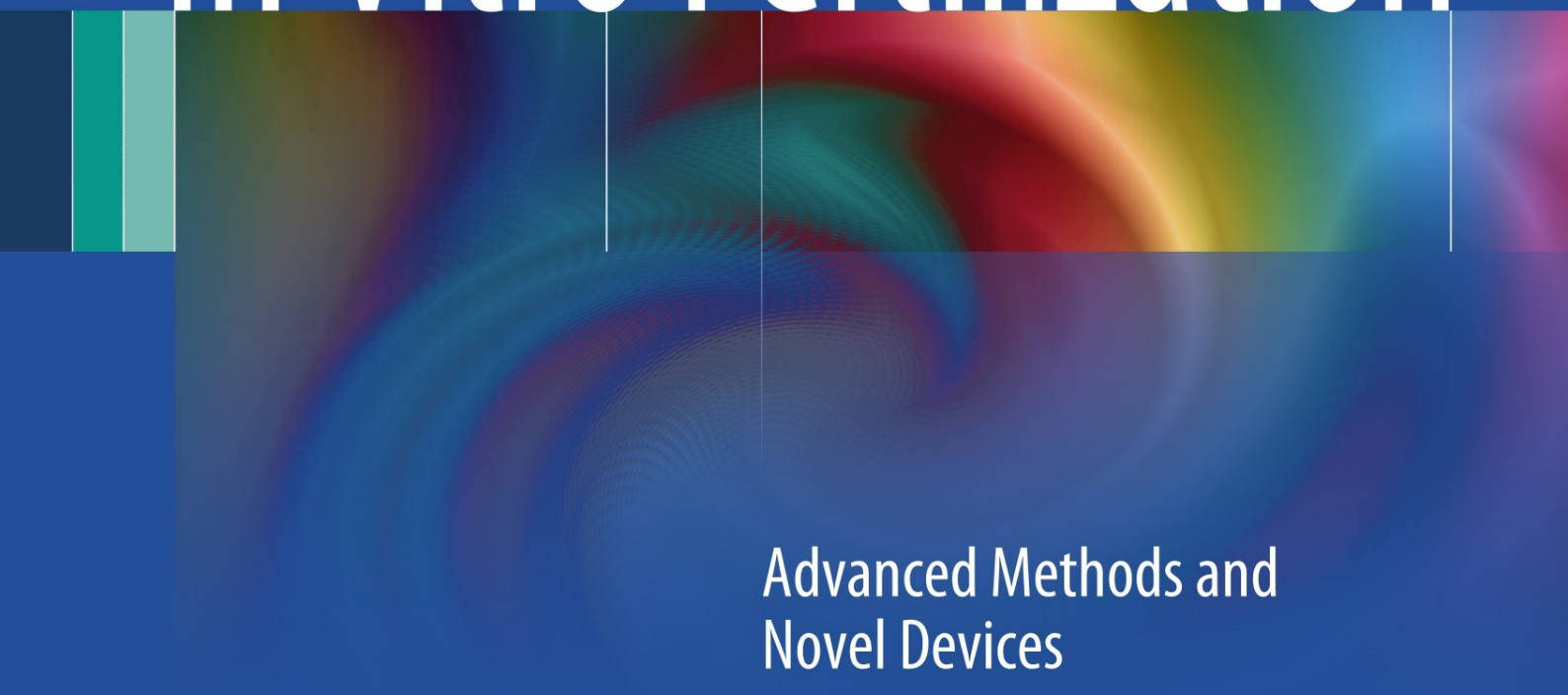
Zsolt Peter Nagy
Alex C. Varghese
Ashok Agarwal
Editors

Practical Manual of In Vitro Fertilization



Advanced Methods and Novel Devices

Springer

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Practical Manual of In Vitro Fertilization

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ISBN 978-1-4419-1779-9 e-ISBN 978-1-4419-1780-5
DOI 10.1007/978-1-4419-1780-5
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2012934564

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Foreword

Even more than 3 decades after the birth of Louise Brown and the birth of several millions of children conceived by numerous procedures of assisted reproductive technology (ART), the *Practical Manual of In Vitro Fertilization*: *Advanced Methods and Novel Devices* is very welcome. The book is edited by Zsolt Peter Nagy, Alex Varghese, and Ashok Agarwal and consists of more than 70 different chapters written by experts in the field. The authors are mostly from North America, but the book includes some experts from Europe and Australia.

The large numbers of chapters are categorized into different major sections: general organization of ART laboratory, the equipment and culture systems used, the characteristics of the oocytes, different procedures of embryo culture, sperm processing and selection, different insemination procedures, the evaluation and grading of embryos, biopsy of oocytes and embryos, cryopreservation of gametes, embryos and tissues, embryo transfer procedures, accreditation and licensing and legislation in different countries. The last part of the *Manual* consists of a series of special topics.

As is the case in all multiauthor books (in this case more than 70 chapters), it is not surprising that there is some diversity in how the different topics are reported. This is the balance between a textbook by one or two authors and a textbook involving not far from two hundred authors. The *Manual* has its place for all involved in the area of reproductive medicine and biology. It is useful for those novices in the field as for those with years of experience. It is especially focused to the ART laboratory which junior and senior embryologists will find very useful. Since ART requires a multidisciplinary approach to be successful, this book has very useful information for all professionals, including reproductive endocrinologists, counselors, nurses, psychologists, etc.

I am convinced that this *Manual* will be of great value for those involved in ART and will be an important aid for all practitioners.

André Van Steirteghem

Preface

In vitro fertilization (IVF) is the most advanced medical technology for the treatment of infertility. During this process, oocytes from the woman and the sperm from the man are brought together outside of the body, in an "artificial" environment (initially using glass made test tubes or Petri dishes and from which the name of the procedure in vitro originates). The first successful application of this technology was in 1978, marked by the birth of world's first "test-tube baby," Louise Brown. Since then, it is estimated that well over four million babies have been born thanks to IVF and thanks to Patrick Steptoe and Robert Edwards. In recognition for this achievement, the Nobel Prize in Physiology or Medicine in 2010 was awarded to Robert G. Edwards. Since the first breakthrough, there have been several significant discoveries and improvements made related to this technology, helping to increase its efficiency several fold.

This textbook has been written with the aim of providing the most comprehensive update on all laboratory aspects of IVF, both theoretical and practical sides, in great detail. In addition, this book also describes several novel techniques that are currently considered experimental, but that in a few years time may become standard procedures.

A total of 75 chapters are included in this book, focused around the following topics: Setting Up and Running an IVF Laboratory; IVF Laboratory Equipment and Culture Systems; In Vitro Fertilization; Embryo Culture Methods; Sperm Processing and Selection; Insemination Procedures; Micromanipulators and Micromanipulation; Embryo Evaluation, Grading, and Assisted Hatching; Biopsy Procedures on Oocytes and Embryos; Cryopreservation; Embryo Transfer; Management and Regulation in the ART Laboratory; and Special Topics. It is of particular interest that these topics were written by the most acclaimed and acknowledged professionals of our field, 184 in total, representing all continents of the world.

Because of the wide range of topics and the comprehensive theoretical and detailed practical descriptions, this book is an ideal reference for all who are involved with assisted reproduction, including embryologists, andrologists, reproductive endocrinologists, and scientists, regardless if one wishes to obtain a basic understanding or a deep, up-to-date presentation.

We would like to thank Richard Lansing, Executive Editor, for his support and advice and Margaret Burns, Developmental Editor, for her enthusiastic and continuous efforts in reviewing and editing each of the manuscripts. Furthermore, we are thankful to all of the outstanding contributors for sharing their knowledge and for being part of this great project. Finally, we are indebted to our families, who provided their support and understanding when time was taken away from them.

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Journey of Human Gametes In Vitro: 1978–2010

1

Zeev Shoham

Abstract

In the 1890s, Walter Heape reported the first known case of embryo transplantation in rabbits. As early as 1932, it was suggested in both fiction and research that human in vitro fertilization (IVF) might be possible. The 1959 birth of rabbits as a result of IVF opened the way to assisted procreation. The field of IVF has developed dramatically during the last 30 years and continues to do so. New drug development, better ovarian stimulation techniques, and improved ways to identify the best embryos will lead to improved pregnancy rates.

Keywords

In vitro fertilization • Ovarian stimulation techniques • Ovarian stimulation protocols • Cryopreservation and storage of oocytes • Embryo transfer

The history of in vitro fertilization (IVF) and embryo transfer (ET) dates back as early as the 1890s when Walter Heape a professor and physician at the University of Cambridge, England, who had been conducting research on reproduction in a number of animal species, reported the first known case of embryo transplantation in rabbits, long before the applications to human fertility were even suggested [1].

In 1932, Aldous Huxley's *Brave New World* was published. In this science fiction novel, Huxley realistically described the technique of IVF as we know it. Two years later Pincus and Enzmann, from the Laboratory of General Physiology at Harvard University, published a paper in the Proceedings of the National Academy of Sciences of the USA, raising the possibility that mammalian eggs can undergo normal development in vitro. However, it was not until 1959 that indisputable evidence of IVF was obtained by Chang [2] who was the first to achieve births in a mammal (a rabbit) by IVF. The newly ovulated eggs were fertilized in vitro by incubation with capacitated sperm in a small Carrel flask for 4 h, thus opening the way to assisted procreation.

Professionals in the fields of microscopy, embryology, and anatomy laid the foundations for future achievements. The recent rapid growth of IVF-ET and related techniques worldwide are further supported by the social and scientific climate which favors their continuation.

Through the years, numerous modifications have been made in the development of IVF-ET in humans: refinement of fertilization and embryo culture media, earlier transfer of the embryo, improvements in equipment, use of a reduced number of spermatozoa in the fertilization dish, and embryo biopsy among others.

The purpose of this manuscript is to acknowledge those who initiated new steps in the development of the treatment protocols and techniques that we now use facilitating such simple and promising IVF-ET procedures.

Development of IVF

In 1965, Edwards together with Georgeanna and Jones at Johns Hopkins Hospital in the United States attempted to fertilize human oocytes in vitro [3]. It took another 8 years until the first IVF pregnancy was reported by the Monash research

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Z.P. Nagy et al. (eds.), *Practical Manual of In Vitro Fertilization: Advanced Methods and Novel Devices*,
DOI 10.1007/978-1-4419-1780-5_1,

team of Professors Carl Wood and John Leeton in Melbourne, Australia. Unfortunately, this resulted in early miscarriage [4]. Another step toward the goal of IVF was noted by Steptoe and Edwards who published a report on an ectopic pregnancy following transfer of a human embryo at the late morulae/early blastocyst stage [5]. However, a breakthrough and a huge achievement was noted in 1978 when the first ever IVF birth occurred in Oldham, England on July 25, 1978. This birth was the result of the collaborative work of Steptoe and Edwards [6]. Following this achievement, a year later, the first IVF birth was noted in Australia by the joint Victorian Monash-Melbourne team which occurred at Royal Women's Hospital [7], following the announcement of Howard and Georgianna Seegar Jones about the delivery of the first IVF baby in the United States in 1981. This first IVF birth in the United States was achieved with the use of hMG for ovarian stimulation. The improvement of technology leads to the report of Jacqueline Mandelbaum with Dan Szollosi describing the microstructures of the human oocyte, which became known as *oocyte dysmorphia* [8].

To improve the results of the procedure, efforts were made in optimizing the stimulation protocols, improving the culture media, improving aspiration technique and holding of the oocytes, and improving fertilization techniques. The second part of this manuscript will describe the fields which were developed in parallel, enabling the improvement of the pregnancy rate. These fields are as follows:

- Stimulation protocols
- Culture media
- Aspiration technique, handling of the oocyte, and embryo replacement
- Improved fertilization
- Cryopreservation and storage of embryos and oocytes
- Future manipulation of the oocytes and embryos

Stimulation Protocols

Earlier IVF pregnancies were achieved from unstimulated ovulatory cycles and the success rate remained low. It was later on suggested that in order to improve the fertilization and pregnancy rate, it might be possible to aspirate more than one egg, and this can be achieved during a cycle in which the ovary is stimulated by endogenous or exogenous gonadotropins. During the years, several protocols were developed and each of it was tested in relation to the drug influence on the oocytes. Trounson et al. introduced the use of clomiphene citrate and hMG in the treatment protocols [9]. It was later demonstrated that GnRH agonists can be used to eliminate premature luteinization and control ovarian stimulation [10].

It was Porter et al. who were the first to use GnRH agonists in IVF treatment [11]. Three major protocols using GnRH agonist were developed: the long, short, and ultra-short protocols. Several years later following numerous studies, it was found that the long protocol gave the best results in terms of pregnancy rate.

The administration of GnRH agonists resulted in "flare-up" of gonadotropins from the hypophysis. In some women, this resulted in the development of follicular cyst. In order to reduce the scale of gonadotropin section following the administration of GnRH agonist, it was suggested to give GnRH agonist either during the luteal phase, when serum progesterone is high, or under progesterone administration or during the oral contraception pill. Gonen, Jacobson, and Casper pioneered the use of combined oral contraceptives for follicle synchronization and cycle scheduling in IVF [12].

Following the development of the various stimulation protocols, it was noted that there is a possibility that the steroids production during the luteal phase was not optimal, and it was Casper et al. who were the first to describe the use of low-dose hCG for support of the luteal phase in ART cycles [13].

In order to simplify the stimulation protocol and to shorten it, efforts were invested in developing the GnRH antagonist. Frydman was the first to report the use of the GnRH antagonist, Nal-Glue, to prevent premature LH rise and progesterone in controlled ovarian hyperstimulation treatment [14]. This specific drug was not developed further due to the side effects. Following further efforts, the third generation of the GnRH antagonist was developed, and the first established pregnancy using recombinant FSH and GnRH antagonist was reported by Itskovitz-Eldor et al. [15]. The increasing needs in infertility treatment created the needs to develop new sources for gonadotropins. Recombinant FSH became available during the year 1992, and very soon after, pregnancy was established using a recombinant drug for ovarian stimulation [16, 17]. Following the development of the technology to produce recombinant FSH, it became only a matter of time until the recombinant LH and hCG became available. Pregnancy after treatment with three recombinant gonadotropins was reported by Agrawal et al. [18].

The above-mentioned development summarizes 30 years of gradual development in drugs production and methods of administration. Several additional developments were noted when Emperaire et al. publish their observation that the final stage of ovulation induction can be induce by endogenous LH released by the administration of an LHRH agonist after follicular stimulation for IVF [19].

In addition, a new technology was developed which involved in vitro maturation (IVM) of the oocyte, and Chian et al. demonstrated that hCG priming prior to immature oocyte retrieval in women with PCO increases the maturation rate and produces high pregnancy rates of 40% per started cycle [20]. It seems today that the various protocols

and the various drugs available for ovarian stimulation enable the treating physicians to individualize treatment to the specific patient.

Culture Media

Optimal culture media which reflects the changing needs of the developing embryo is of importance for success of IVF treatment. The environment of the oviduct/uterus change dramatically as the zygote/embryo travels. The culture media needs to meet the metabolic needs of preimplantation embryos by addressing energetic and amino acid requirements in a stage-specific manner.

The first advanced culture medium was introduced in 1980 [21]. In 1985, Quinn and Warnes published a formula entitled human tubal fluid (HTF) that mimics the in vivo environment to which the embryo is exposed [22]. Gardner introduced sequential media and blastocyst transfer which now greatly assists in the move to single embryo transfer [23, 24].

The development of a completely chemically defined protein-free embryo culture medium and the births of the first batch of babies generated from the fertilization of eggs collected and inseminated in the medium using spermatozoa also prepared in the same protein-free medium in both conventional IVF and intracytoplasmic sperm injection (ICSI) were introduced in 2000 [25], and since then, the medium remained almost the same.

Aspiration Technique and Handling of the Oocyte and Embryo Replacement

Several techniques for oocyte aspiration have been developed to obtain the largest number of mature oocytes with the least risk to the patients causing minimal damage to the ovary. This was started with laparoscopic retrieval introduced by Steptoe and Edwards in 1978, and laparotomy for infertility related conditions [26].

Lenz and Lauritsen examined and proposed the transabdominal transvesical oocyte aspiration using an ultrasound-guided needle [27]. A year later, Gleicher and his group reported the first vaginal egg retrieval using an abdominal ultrasound [28].

Wikland, together with Hamberger and Nilsson in Gothenburg, Sweden, described the possibility of using a vaginal sector scanner (transvesical or transvaginal technique) for oocyte aspiration [29]. At the same time, Strickler et al. [30] described embryo transfer using abdominal ultrasound guidance. This technique gained additional support from the work done by Feichtinger and Kemeter [31] and it remained the main technique for egg collection until today.

Improved Fertilization

In the process of fertilization, it was first reported by Trounson and his group that there is a need for a delay between oocyte collection and insemination to allow oocytes collected to complete maturation [32]. In parallel, the possibility of using the natural environment of the human tube for the process of fertilization and development of the embryo lead to the development of the gamete intrafallopian tube (GIFT) procedure, and it was Asch et al., in 1984, who were the first to report on pregnancy following translaparoscopic GIFT procedure [33].

Much effort was invested trying to improve fertilization when this would not occur naturally when the egg and the sperm where introduced at the Petri dish in the laboratory. These efforts were started when researchers try to fertilize human oocytes by microinjection of a single sperm under the zona pellucida [34] which was followed by the report of a health delivery using the same technique by Ng et al. [35]. Further achievement was noted when pregnancy was obtained from micromanipulation using zona drilling or mechanical partial zona dissection [36]. However, a breakthrough was achieved when Palermo et al. from the group in Brussels reported on the first pregnancy after ICSI [37].

In order to improve fertilization results after repeated IVF failure or at advanced maternal age, it was suggested to use the cytoplasmic transfer technique. This is based on an assumption that a vital molecule, such as ATP or cell cycle-related kinase, or an organelle, such as mitochondria, is deficient at a critical stage in early development.

The technique aims to improve egg and embryo quality by the donation of ooplasm from supposedly fertile oocytes to patients whose cells are of poorer quality. Transfer of anucleate cytoplasm has been used, and Choen et al. [38], was the first to report on a birth after such a procedure.

Development of Cryopreservation and Storage of Embryos and Oocytes

In 1983, the group of investigators at the Monash IVF Centre reported on the first human pregnancy and birth following cryopreservation, thawing, and transfer of an eight-cell embryo. This embryo-freezing technique was developed in Cambridge, England, on cattle, and with minor adaptations, it was adjusted to humans [39]. A few years later, in 1990, Gordyts et al. reported on the first successful human cleavage-stage embryo vitrification followed by a successful delivery [40]. However, until this period of time, there were difficulties to cryopreserved oocytes, and only in 1999, the birth following vitrification of human oocyte was reported by Kuleshova et al. [41]. During the same year, Porcu et al. reported on the first birth from cryopreserved oocytes and

testicular sperm [42]. A major development was noted when Bedaiwy et al. reported in 2006 on the successful cryopreservation of intact human ovary with its vascular pedicle [43]. This was followed in 2008 by the announcement of Porcu et al. on cryopreserved oocytes in cancer patients and the first ever birth of healthy twins after oocyte cryopreservation and bilateral ovariectomy [44].

Manipulation of the IVF Procedure and Technique to Solve Various Causes of Infertility

In 1983, the Monash IVF team achieved the first birth in a woman without ovaries by using donor eggs, by creating of artificial menstrual cycles, and by using a special hormonal formula for the first 10 weeks of pregnancy [45]. This came along with the first successful delivery following egg donation [46]. Still at the same year, in vitro maturation and -fertilization of morphologically immature human oocytes in an IVF setup were developed and published [47]. However, it took 11 years until the first live birth as a result of IVM following transvaginal ultrasound-guided oocyte collection was reported. Further development of the technique was reported by Cohen et al. who achieved a blastocyst development from IVM oocyte plus ICSI plus assisted hatching, which was ended in a healthy birth [48].

The role of assisted hatching is still controversial, and the indication for the procedure is not very clear. However, in 1985, Cohen et al. reported in the Lancet about a birth after replacement of hatching blastocyst cryopreserved at the expanded blastocyst stage [49]. It was later on suggested to use this specific technique of assisted zona hatching to breach the zona pellucida and promote the natural process of hatching when the prognosis is poor [50].

A new era in the field of IVF, i.e., preimplantation genetic diagnosis (PGD) was open with the announcement of Handyside et al. first reported on biopsy of human preimplantation embryos and sexing by DNA amplification [51]. This was followed by the report of pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification [52]. Still in the same field of PGD, Verlinsky was the first to report on the possibility to do a polar body biopsy, transfer the embryo, and achieve pregnancy [53].

Summary

The field of IVF was developed dramatically during the last 30 years and what was considered to be a miracle has become a common practice around the world. The field is still being developed in various directions to find solution to those couples who are infertile, to those who wish to become pregnant at an elder age, beyond the boundaries of nature, and to those who, due to medical reasons, need to delay the time of procreation.

British physiologist Robert Edwards, whose work led to the first "test-tube baby", won the 2010 Nobel prize for medicine or physiology. As many as 4 million babies have been born since the first IVF baby in 1978 as a result of the techniques Edwards developed, together with a now-deceased colleague, Patrick Steptoe.

We can expect further developments in the drug development, reduce the difficulties involved in stimulation of the ovaries moving into natural cycles, and obviously find a way to define the best embryo for replacement which might give the patients the highest chance to become pregnant.

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