Textbook of Assisted Reproductive Techniques

Volume 1&2: CLINICAL PERSPECTIVES

Edited by
David K. Gardner
Ariel Weissman
Colin M. Howles
Zeev Shoham
The editors would like to dedicate this edition to the late Professor Robert G. Edwards and the late Queenie V. Neri.

The editors (from left to right: Ariel Weissman, David K Gardner, Zeev Shoham, Colin M Howles) at the annual meeting of ESHRE, Geneva, July 2017.
# Contents

## Contributors
- The beginnings of human in vitro fertilization
  - Robert G. Edwards
  - Robert G. Edwards and the thorny path to the birth of Louise Brown: A history of in vitro fertilization and embryo transfer
    - Martin H. Johnson

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>New guidelines for setting up an assisted reproduction technology laboratory</td>
</tr>
<tr>
<td></td>
<td>Jacques Cohen, Mina Alikani, Antonia Gilligan, and Tim Schimmel</td>
</tr>
<tr>
<td>2</td>
<td>Quality control: Maintaining stability in the laboratory</td>
</tr>
<tr>
<td></td>
<td>Ronny Janssens and Johan Guns</td>
</tr>
<tr>
<td>3</td>
<td>The assisted reproduction technology laboratory: Current standards</td>
</tr>
<tr>
<td></td>
<td>Cecilia Sjöblom</td>
</tr>
<tr>
<td>4</td>
<td>Evaluation of sperm</td>
</tr>
<tr>
<td></td>
<td>Kaylen M. Silverberg and Tom Turner</td>
</tr>
<tr>
<td>5</td>
<td>Sperm preparation techniques</td>
</tr>
<tr>
<td></td>
<td>Harold Bourne and Janell Archer</td>
</tr>
<tr>
<td>6</td>
<td>Sperm chromatin assessment</td>
</tr>
<tr>
<td></td>
<td>Ashok Agarwal, Rakesh Sharma, and Gulfam Ahmad</td>
</tr>
<tr>
<td>7</td>
<td>Oocyte retrieval and selection</td>
</tr>
<tr>
<td></td>
<td>Laura F. Rienzi and Filippo M. Ubaldi</td>
</tr>
<tr>
<td>8</td>
<td>Preparation and evaluation of oocytes for intracytoplasmic sperm injection</td>
</tr>
<tr>
<td></td>
<td>Irit Granot and Nava Dekel</td>
</tr>
<tr>
<td>9</td>
<td>Advanced sperm selection techniques for intracytoplasmic sperm injection</td>
</tr>
<tr>
<td></td>
<td>Tamer M. Said, Reda Z. Mahfouz, and Alfonso P. Del Valle</td>
</tr>
<tr>
<td>10</td>
<td>Use of in vitro maturation in a clinical setting: Patient populations and outcomes</td>
</tr>
<tr>
<td></td>
<td>Yoshiharu Morimoto, Aisaku Fukuda, and Manabu Satou</td>
</tr>
<tr>
<td>11</td>
<td>Intracytoplasmic sperm injection: Technical aspects</td>
</tr>
<tr>
<td></td>
<td>Queenie V. Neri, Nigel Pereira, Tyler Cozzubbo, Zev Rosenwaks, and Gianpiero D. Palermo</td>
</tr>
<tr>
<td>12</td>
<td>Assisted hatching</td>
</tr>
<tr>
<td></td>
<td>Anna Veiga and Itziar Belil</td>
</tr>
<tr>
<td>13</td>
<td>Human embryo biopsy procedures</td>
</tr>
<tr>
<td></td>
<td>Jason Kofinas, Caroline McCaffrey, and James Grifo</td>
</tr>
<tr>
<td>14</td>
<td>Assisted oocyte activation: Current understanding, practice, and future perspectives</td>
</tr>
<tr>
<td></td>
<td>Junaid Kashir and Karl Swann</td>
</tr>
<tr>
<td>15</td>
<td>Analysis of fertilization</td>
</tr>
<tr>
<td></td>
<td>Thomas Ebner</td>
</tr>
<tr>
<td>16</td>
<td>Culture systems for the human embryo</td>
</tr>
<tr>
<td></td>
<td>David K. Gardner and Michelle Lane</td>
</tr>
<tr>
<td>17</td>
<td>Evaluation of embryo quality: Analysis of morphology and physiology</td>
</tr>
<tr>
<td></td>
<td>Denny Sakkas and David K. Gardner</td>
</tr>
<tr>
<td>18</td>
<td>Evaluation of embryo quality: Time-lapse imaging to assess embryo morphokinesis</td>
</tr>
<tr>
<td></td>
<td>Natalia Basile, Andrea Rodrigo Carbajosa, and Marcos Meseguer</td>
</tr>
<tr>
<td>19</td>
<td>Evaluation of embryo quality: Proteomic strategies</td>
</tr>
<tr>
<td></td>
<td>Mandy Katz-Jaffe</td>
</tr>
<tr>
<td>20</td>
<td>The human oocyte: Controlled-rate cooling</td>
</tr>
<tr>
<td></td>
<td>Carlotta Zacà and Andrea Borini</td>
</tr>
<tr>
<td>21</td>
<td>The human oocyte: Vitrification</td>
</tr>
<tr>
<td></td>
<td>Masashige Kuwayama</td>
</tr>
<tr>
<td>22</td>
<td>The human embryo: Slow freezing</td>
</tr>
<tr>
<td></td>
<td>Marius Meintjes</td>
</tr>
<tr>
<td>23</td>
<td>The human embryo: Vitrification</td>
</tr>
<tr>
<td></td>
<td>Zsolt Peter Nagy, Ching-Chien Chang, and Gábor Vajta</td>
</tr>
</tbody>
</table>
24 Managing an oocyte bank
Ana Cobo, Pilar Alamá, José María de los Santos, María José de los Santos, and José Remohí 316

25 Severe male factor infertility: Genetic consequences and recommendations for genetic testing
Katrien Stouffs, Willy Lissens, and Sara Seneca 326

26 Polar body biopsy and its clinical application
Markus Montag 339

27 Preimplantation genetic diagnosis for infertility
Jonathan Lewin and Dagan Wells 350

28 Genetic analysis of the embryo
Yuval Yaron, Liran Hiersch, Veronica Gold, Sagit Peleg-Schalka, and Mira Malcov 359

29 Diagnosis of endometrial receptivity and the embryo–endometrial dialog
Francisco Domínguez, Maria Ruiz-Alonso, Felipe Vilella, and Carlos Simón 373

30 Artificial gametes: The oocyte
Evelyn E. Telfer and Kelsey M. Grieve 381

31 Microfluidics in assisted reproduction technology: Towards automation of the in vitro fertilization laboratory
Jason E. Swain 390

32 Epigenetic considerations in preimplantation mammalian embryos
Heide Schatten and Qing-Yuan Sun 410

Index 415
Contributors

Ashok Agarwal  
American Center for Reproductive Medicine  
Cleveland Clinic  
Cleveland, Ohio

Gulfam Ahmad  
American Center for Reproductive Medicine  
Cleveland Clinic  
Cleveland, Ohio  
and  
Department of Physiology  
University of Health Sciences  
Lahore, Pakistan  
and  
College of Medicine  
Prince Sattam bin Abdulaziz University  
AlKharj, Kingdom of Saudi Arabia

Pilar Alamá  
IVI  
Valencia, Spain

Mina Alikani  
Tyho-Galileo Research Laboratories  
Livingston, New Jersey

Janell Archer  
Reproductive Services/Melbourne IVF  
Royal Women’s Hospital  
Melbourne, Australia

Natalia Basile  
IVI  
Madrid, Spain

Itziar Belil  
Reproductive Medicine Service  
Hospital Universitari Dexeus  
Barcelona, Spain

Andrea Borini  
9.baby—Family and Fertility Center  
Bologna, Italy

Harold Bourne  
Reproductive Services/Melbourne IVF  
Royal Women’s Hospital  
Melbourne, Australia

Andrea Rodrigo Carbajosa  
Clínica Tambre  
Madrid, Spain

Ching-Chien Chang  
Reproductive Biology Associates  
Atlanta, Georgia

Ana Cobo  
IVI  
Valencia, Spain

Jacques Cohen  
ART Institute of Washington  
and  
Tyho-Galileo Research Laboratories  
Livingston, New Jersey

Tyler Cozzubbo  
The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine  
Weill Cornell Medical College  
New York City, New York

Nava Dekel  
Department of Biological Regulation  
The Weizmann Institute of Science  
Rehovot, Israel

Alfonso P. Del Valle  
The Toronto Institute for Reproductive Medicine—ReproMed  
Toronto, Canada

Francisco Domínguez  
Valencia University  
INCLIVA  
Valencia, Spain

Thomas Ebner  
Kepler University  
Kinderwunsch Zentrum  
Linz, Austria

Robert G. Edwards (Deceased)  
Duck End Farm  
Cambridge, United Kingdom

Aisaku Fukuda  
IVF Osaka Clinic  
Higashi-Osaka, Osaka, Japan

David K. Gardner  
School of BioSciences  
University of Melbourne  
and  
Melbourne IVF  
Victoria, Australia
Antonia Gilligan  
Alpha Environmental  
Livingston, New Jersey

Veronica Gold  
Sara Racine In Vitro Fertilization Unit  
Tel Aviv Sourasky Medical Center  
Tel Aviv, Israel

Irit Granot  
IVF Unit  
Herzliya Medical Center  
Herzliya, Israel

Kelsey M. Grieve  
Institute of Cell Biology and Centre for Integrative Physiology  
and  
MRC Centre for Reproductive Health  
Queen’s Medical Research Institute  
University of Edinburgh  
Edinburgh, United Kingdom

James Grifo  
Department of Obstetrics and Gynecology  
NYU Langone Medical Center  
New York City, New York

Johan Guns  
Centre for Reproductive Medicine  
UZ Brussel  
Brussels, Belgium

Liran Hiersch  
Department of Obstetrics and Gynecology  
Lis Maternity Hospital  
Tel Aviv Sourasky Medical Center  
and  
Sackler Faculty of Medicine  
Tel Aviv University  
Tel Aviv, Israel

Ronny Janssens  
Centre for Reproductive Medicine  
UZ Brussel  
Brussels, Belgium

Martin H. Johnson  
Department of Physiology, Development & Neuroscience  
School of Anatomy  
University of Cambridge  
and  
Christ’s College  
Cambridge, United Kingdom

María José de los Santos  
IVI, Valencia, Spain

Junaid Kashir  
College of Biomedical and Life Sciences  
Cardiff University  
Cardiff, United Kingdom  
and  
College of Medicine  
Alfaisal University  
and  
Department of Comparative Medicine  
King Faisal Specialist Hospital and Research Centre  
Riyadh, Saudi Arabia

Mandy Katz-Jaffe  
Colorado Center for Reproductive Medicine  
Lone Tree, Colorado

Jason Kofinas  
Kofinas Fertility Group  
New York City, New York

Masashige Kuwayama  
Repro-Support Medical Research Centre  
ReproLife, Inc.  
Tokyo, Japan

Michelle Lane  
Department of Obstetrics and Gynecology  
University of Adelaide  
Adelaide, Australia  
and  
Repromed  
Dulwich, Australia

Jonathan Lewin  
Nuffield Department of Obstetrics and Gynaecology  
University of Oxford  
Oxford, United Kingdom

Willy Lissens  
Center for Medical Genetics/Research Center Reproduction and Genetics  
Vrije Universiteit Brussel (VUB)  
Universitair Ziekenhuis Brussel (UZ Brussel)  
Brussels, Belgium

Reda Z. Mahfouz  
THOR Department  
Cleveland Clinic Lerner College of Medicine  
Case Western Reserve University  
Cleveland, Ohio

Mira Malcov  
Sara Racine In Vitro Fertilization Unit  
Tel Aviv Sourasky Medical Center  
Tel Aviv, Israel
José Maria de los Santos
IVI
Valencia, Spain

Caroline McCaffrey
Department of Obstetrics and Gynecology
NYU Langone Medical Center
New York City, New York

Marius Meintjes
Frisco Institute for Reproductive Medicine
Frisco, Texas

Marcos Meseguer
IVI Valencia
Valencia, Spain

Markus Montag
ilabcomm GmbH
Sankt Augustin, Germany

Yoshiharu Morimoto
HORAC Grand Front Osaka Clinic
Osaka City, Japan

Zsolt Peter Nagy
Reproductive Biology Associates
Atlanta, Georgia

Queenie V. Neri (Deceased)
The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine
Weill Cornell Medical College
New York, New York

Gianpiero D. Palermo
The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine
Weill Cornell Medical College
New York, New York

Sagit Peleg-Schalka
Sara Racine In Vitro Fertilization Unit,
Tel Aviv Sourasky Medical Center
Tel Aviv, Israel

Nigel Pereira
The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine
Weill Cornell Medical College
New York, New York

José Remohí
IVI
Valencia, Spain

Laura F. Rienzi
GENERAL Centres for Reproductive Medicine
Roma, Marostica, Umbertide, Napoli, Italy

Zev Rosenwaks
The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine
Weill Cornell Medical College
New York City, New York

Maria Ruiz-Alonso
Igenomix
Valencia, Spain

Tamer M. Said
The Toronto Institute for Reproductive Medicine—ReproMed
Toronto, Canada

Denny Sakkas
Boston IVF
Waltham, Massachusetts

Manabu Satou
IVF Namba Clinic
Nishi-ku, Osaka, Japan

Heide Schatten
Department of Veterinary Pathobiology
University of Missouri
Columbia, Missouri

Tim Schimmel
ART Institute of Washington
and
Tyho-Galileo Research Laboratories
Livingston, New Jersey

Sara Seneca
Center for Medical Genetics/Research Center
Reproduction and Genetics
Vrije Universiteit Brussel (VUB)
Universitair Ziekenhuis Brussel (UZ Brussel)
Brussels, Belgium

Rakesh Sharma
American Center for Reproductive Medicine
Cleveland Clinic
Cleveland, Ohio

Kaylen M. Silverberg
Texas Fertility Center
Ovation Fertility
Austin, Texas

Carlos Simón
Igenomix
Valencia, Spain
Contributors

Cecilia Sjöblom
Westmead Fertility Centre
Institute of Reproductive Medicine
University of Sydney
Westmead, Australia

Katrien Stouffs
Center for Medical Genetics/Research Center
Reproduction and Genetics
Vrije Universiteit Brussel (VUB)
Universitair Ziekenhuis Brussel (UZ Brussel)
Brussels, Belgium

Qing-Yuan Sun
State Key Laboratory of Reproductive Biology
Institute of Zoology
Chinese Academy of Sciences
Beijing, China

Jason E. Swain
CCRM IVF Network
Lone Tree, Colorado

Karl Swann
College of Biomedical and Life Sciences
Cardiff University
Cardiff, United Kingdom

Evelyn E. Telfer
Institute of Cell Biology and Centre for Integrative Physiology
University of Edinburgh
Edinburgh, United Kingdom

Tom Turner
Texas Fertility Center
Ovation Fertility
Austin, Texas

Filippo M. Ubaldi
GENER A Centres for Reproductive Medicine
Rome, Italy

Gábor Vajta
RVT Australia
Queensland, Australia

Anna Veiga
Reproductive Medicine Service
Hospital Universitari Dexeus
and
Barcelona Stem Cell Bank
Centre of Regenerative Medicine in Barcelona
Barcelona, Spain

Felipe Vilella
Igenomix
Valencia, Spain

Dagan Wells
Nuffield Department of Obstetrics & Gynaecology
University of Oxford
Oxford, United Kingdom

Yuval Yaron
Prenatal Genetic Diagnosis Division, Genetic Institute
and
Department of Obstetrics and Gynecology
Lis Maternity Hospital
Tel Aviv Sourasky Medical Center
and
Sackler Faculty of Medicine
Tel Aviv University
Tel Aviv, Israel

Carlotta Zacà
9.baby—Family and Fertility Center
Bologna, Italy
The beginnings of human *in vitro* fertilization

ROBERT G. EDWARDS

*In vitro* fertilization (IVF) and its derivatives in preimplantation diagnosis, stem cells, and the ethics of assisted reproduction continue to attract immense attention scientifically and socially. All these topics were introduced by 1970. Hardly a day passes without some public recognition of events related to this study, and clinics spread ever further worldwide. Now that we must be approaching 1.5 million IVF births, it is time to celebrate what has been achieved by so many investigators, clinical, scientific, and ethical.

While much of this “Introduction” chapter covers the massive accumulation of events between 1960 and 2000, it also briefly discusses new perspectives emerging in the twenty-first century. Fresh advances also increase curiosity about how these fields of study began and how their ethical implications were addressed in earlier days. As for me, I am still stirred by recollections of those early days. Foundations were laid in Edinburgh, London, and Glasgow in the 1950s and early 1960s. Discoveries made then led to later days in Cambridge, working there with many PhD students. It also resulted in my working with Patrick Steptoe in Oldham. Our joint opening of Bourn Hall in 1980, which became the largest IVF clinic of its kind at the time, signified the end of the beginning of assisted human conception and the onset of dedicated applied studies.

**INTRODUCTION**

First of all, I must express in limited space my tributes to my teachers, even if inadequately. These include investigators from far-off days when the fundamental facts of reproductive cycles, surgical techniques, endocrinology, and genetics were elicited by many investigators. These fields began to move in the twentieth century, and if one pioneer of these times should be saluted, it must be Gregory Pincus. Famous for the contraceptive pill, he was a distinguished embryologist, and part of his work dealt with the maturation of mammalian oocytes *in vitro*. He was the first to show how oocytes aspirated from their follicles would begin their maturation *in vitro*, and how a number matured and expelled a first polar body. I believe his major work was done in rabbits, where he found that the 10–11-hour timings of maturation *in vitro* accorded exactly with those occurring *in vivo* after an ovulatory stimulus to the female rabbit.

Pincus et al. also studied human oocytes (1). Extracting oocytes from excised ovaries, they identified chromosomes in a large number of oocytes and interpreted this as evidence of the completion of maturation *in vitro*. Many oocytes possessed chromosomes after 12 hours, with the proportion remaining constant over the next 30 hours and longer. Twelve hours was taken as the period of maturation. Unfortunately, chromosomes were not classified for their meiotic stage. Maturing oocytes would be expected to display diakinesis or metaphase I chromosome pairs. Fully mature oocytes would display metaphase II chromosomes, signifying they were fully ripe and ready for fertilization. Nevertheless, it is well known that oocytes can undergo atresia in the ovary, involving the formation of metaphase II chromosomes in many of them. These oocytes complicated Pincus’ estimates, even in controls, and were the source of his error, which led later workers to inseminate human oocytes 12 hours after collection and culture *in vitro* (2,3). Work on human fertilization *in vitro*, and indeed comparable studies in animals, remained in abeyance from then and for many years. Progress in animal IVF had also been slow. After many relatively unsuccessful attempts in several species in the 1950s and 1960s, a virtual dogma arose that spermatozoa had to spend several hours in the female reproductive tract before acquiring the potential to bind to the zona pellucida and achieve fertilization. In the late 1960s, Austin and Chang independently determined the need for sperm capacitation, identified by a delay in fertilization after spermatozoa had entered the female reproductive tract (4,5). This discovery was taken by many investigators as the reason for the failure to achieve fertilization *in vitro*, and why spermatozoa had to be exposed to secretions of the female reproductive tract. At the same time, Chang reported that rabbit eggs that had fully matured *in vitro* failed to produce normal blastocysts, with none of them implanting normally (6).

**MODERN BEGINNINGS OF HUMAN IVF, PREIMPLANTATION GENETIC DIAGNOSIS, AND EMBRYO STEM CELLS**

My PhD began at the Institute of Animal Genetics, Edinburgh University, in 1952, encouraged by Professor Conrad Waddington, the inventor of epigenesis, and supervised by Dr. Alan Beatty. At the time, capacitation was gaining in significance. My chosen topic was the genetic control of early mammalian embryology, specifically the growth of preimplantation mouse embryos with altered chromosome complements.

Achieving these aims included a need to expose mouse spermatozoa to x-rays, ultraviolet light, and various chemicals *in vitro*. This would destroy their chromatin and prevent them from making any genetic contribution.
to the embryo, hopefully without impairing their capacity to fertilize eggs in vivo. Resulting embryos would become gynogenetic haploids. Later, my work changed to exposing ovulated mouse oocytes to colchicine in vivo in order to destroy their second meiotic spindle in vivo. This treatment freed all chromosomes from their attachment to the meiotic spindle, and they then became extruded from the egg into tiny artificial polar bodies. The fertilizing spermatozoon thus entered an empty egg, which resulted in the formation of androgenetic haploid embryos with no genetic contribution from the maternal side. For three years, my work was concentrated in the mouse house, working at midnight to identify mouse females in estrus by vaginal smears, collecting epididymal spermatozoa from males, and practicing artificial insemination with samples of treated spermatozoa. This research was successful, as mouse embryos were identified with haploid, triploid, tetraploid, and aneuploid chromosomes. Moreover, the wide range of scientific talent in the Institute made it a perfect place for fresh collaborative studies. For example, Julio Sirlin and I applied the use of radioactive DNA and RNA precursors to the study of spermatogenesis, spermiogenesis, fertilization, and embryogenesis, and gained knowledge unavailable elsewhere.

An even greater fortune beckoned. Allen Gates, who was newly arrived from the United States, brought commercial samples of Organon's pregnant mares' serum (PMS) rich in follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG) with its strong luteinizing hormone (LH) activity to induce estrus and ovulation in immature female mice. Working with Mervyn Runner (7), he had used low doses of each hormone at an interval of 48 hours to induce oocyte maturation, mating, and ovulation in immature mouse females. He now wished to measure the viability of three-day embryos from immature mice by transferring them to an adult host to grow to term (8). I was more interested in stimulating adult mice with these gonadotropins to induce estrus and ovulation at predictable times of the day. This would help my research, and I was by now weary of taking mouse vaginal smears at midnight. My future wife, Ruth Fowler, and I teamed up to test this new approach to superovulating adult mice. We chose PMS to induce multil folliculation and hCG to trigger ovulation, varying the doses and times from those utilized by Allen Gates. PMS became obsolete for human studies some time later, but its impact has stayed with me from that moment, even until today.

Opinion in those days was that exogenous hormones such as PMS and hCG would stimulate follicle growth and ovulation in immature female mammals, but not in adults because they would interact badly with an adult’s reproductive cycles. In fact, they worked wonderfully well. Doses of 1–3 IU of PMS induced the growth of numerous follicles, and similar doses of hCG 42 hours later invoked estrus and ovulation a further 6 hours later in almost all of them. Often, 70 or more ovulated oocytes crowded the ampulla, most of them being fertilized and developing to blastocysts (9). Oocyte maturation, ovulation, mating, and fertilization were each closely timed in all adults, another highly unusual aspect of stimulation (10). Diakinesis was identified as the germinal vesicle regressed, with metaphase I a little later and metaphase II—expulsion of the first polar body—and ovulation at 11.5–12 hours after hCG. Multiple fertilization led to multiple implantation and fetal growth to full term, just as similar treatments in anovulatory women resulted in quintuplets and other high-order multiple pregnancies a few years later. Years afterward, germinal vesicle breakdown and diakinesis were to prove equally decisive in identifying meiosis and ovulation in human oocytes in vivo and in vitro. Even as these results were gained, Ruth and I departed in 1957 from Edinburgh to the California Institute of Technology, where I switched over to immunology and reproduction, a topic that was to dominate my life for five or six years on my return to the United Kingdom.

The Institute at Edinburgh had given me an excellent basis not only in genetics, but equally in reproduction. I had gained considerable knowledge about the endocrine control of estrus cycles, ovulation, and spermatozoa; the male reproductive tract; artificial insemination; the stages of embryo growth in the oviduct and uterus; superovulation and its consequences; and the use of radiolabeled compounds. Waddington had also been deeply interested in ethics and the relationships between science and religion, and instilled these topics in his students. I had been essentially trained in reproduction, genetics, and scientific ethics, and all of this knowledge was to prove to be of immense value in my later career. A visit to the California Institute of Technology widened my horizons into the molecular biology of DNA and the gene, a field then in its infancy.

After a year in California, London beckoned me to the National Institute for Medical Research to work with Drs. Alan Parkes and Colin (Bunny) Austin. I was fortunate indeed to have two such excellent colleagues. After two intense years in immunology, my curiosity returned to maturing oocytes and fertilization in vitro. Since they matured so regularly and easily in vivo, it should be easy to stimulate maturation in mouse oocytes in vitro by using gonadotropins. In fact, to my immense surprise, when liberated from their follicles into culture medium, oocytes matured immediately in vast numbers in all groups, with exactly the same timing as those maturing in vivo following an injection of hCG. Adding hormones made no difference. Rabbit, hamster, and rat oocytes also matured within 12 hours, each at their own species’ specific rates. But to my surprise, oocytes from cows, sheep, and rhesus monkeys, and the occasional baboon, did not mature in vitro within 12 hours. Their germinal vesicles persisted unmoved, arrested in the stage known as diffuse diplotene. Why had they not responded like those of rats, mice, and rabbits? How would human oocytes respond? A unique opportunity emerged to collect pieces of human ovary and to aspirate human oocytes from their occasional follicles. I grasped it with alacrity.
MOVING TO HUMAN STUDIES

Molly Rose was a local gynecologist in the Edgware and District Hospital who delivered two of our daughters. She agreed to send me slivers or wedges of ovaries such as those removed from patients with polycystic disease, as recommended by Stein and Leventhal, or with myomata or other disorders demanding surgery. Stein–Leventhal wedges were the best sources of oocytes, with their numerous small Graafian follicles lined up in a continuous rim just below the ovarian surface. Though samples were rare, they provided enough oocytes to start with. These oocytes responded just like the oocytes from cows, sheep, and pigs, their germinal vesicles persisting and diakinesis being absent after 12 hours in vitro.

This was disappointing, and especially so for me, since Tjio, Levan, and Ford had identified 46 diploid chromosomes in humans, while studies by teams in Edinburgh and France had made it clear that many human beings were heteroploid. This was my subject, because chromosomal variations mostly arose during meiosis, and this would be easily assessed in maturing oocytes at diakinesis. Various groups also discovered monosomy or disomy in many men and women. Some women were XO or XXX; some men were XYY and XYYY. Trisomy 21 proved to be the most common cause of Down’s syndrome, and other trisomies were detected. All this new information reminded me of my chromosome studies in the Edinburgh mice.

For human studies, I would have to obtain diakinesis and metaphase I in human oocytes, and then continue this analysis to metaphase II when the oocytes would be fully mature, ready for fertilization. Despite being disappointed at the current failure with human oocytes, it was time to write my findings for Nature in 1962 (11). There was so much to write regarding the animal work and in describing the new ideas then taking shape in my mind. I had heard Institute lectures on infertility, and realized that fertilizing human oocytes in vitro and replacing embryos into the mother could help to alleviate this condition. It could also be possible to type embryos for genetic diseases when a familial disposition was identified. Pieces of tissue, or one or two blastomeres, would have to be excised from blastocysts or cleaving embryos, but this did not seem to be too difficult. There were few genetic markers available for this purpose in the early 1960s, but it might be possible to sex embryos by their XX or XY chromosome complement by assessing mitoses in cells excised from morulae or blastocysts. Choosing female embryos for transfer would avert the birth of boys with various sex-linked disorders such as hemophilia. Clearly, I was becoming totally committed to human IVF and embryo transfer.

While looking in the library for any newly published papers relevant to my proposed Nature manuscript, I discovered those earlier papers of Pincus and his colleagues. They had apparently succeeded 30 years earlier in maturing human oocytes cultured for 12 hours where I had failed. My Nature paper (11) became very different from that originally intended, even though it retained enough for publication. Those results of Pincus et al. had to be repeated. After trying hard, I failed completely to repeat them, despite infusing intact ovaries in vitro with gonadotropin solutions, using different culture media to induce maturation, and using joint cultures of maturing mouse oocytes and newly released human oocytes. Adding hormones to culture media also failed. It began to seem that menstrual cycles had affected oocyte physiology in a different manner than in non-menstruating mammalian species. Finally, another line of inquiry emerged after two years of fruitless research on the precious few human oocytes available. Perhaps the timing of maturation in mice and rabbits differed from that of those oocytes obtained from cows, baboons, and humans. Even as my days in London were ending, Molly Rose sent a sliver of human ovary. The few oocytes were placed in culture just as before. Their germinal vesicles remained static for 12 hours as I already knew, and then, after 20 hours in vitro, three oocytes remained, and I waited to examine them until they had been in vitro for 24 hours. The first contained a germinal vesicle, and so did the second. There was one left and one only. Its image under the microscope was electrifying. I gazed down at chromosomes in diakinesis and at a regressing germinal vesicle. The chromosomes were superb examples of human diakinesis with their classical chiasmata. At last, I was on the way to human IVF, to completion of the maturation program and the onset of studies on fertilization in vitro.

This was the step I had waited for, a marker that Pincus had missed. He never checked for diakinesis, and apparently confused atretic oocytes, which contained chromosomes, with maturing oocytes. Endless human studies were opening. It was easy now, even on the basis of one oocyte in diakinesis, to calculate the timing of the final stages of maturation because the post-diakinesis stages of maturation were not too different from normal mitotic cycles in somatic cells. This calculation provided me with an estimate of about 36 hours for full maturation, which would be the moment for insemination. All these gaps in knowledge had to be filled. But now, my research program was stretching far into the future.

At this wonderful moment, John Paul, an outstanding cell biologist, invited me to join him and Robin Cole at Glasgow University to study differentiation in early mammalian embryos. This was exciting, to work in biochemistry with a leading cell biologist. He had heard that I was experimenting with very early embryos, trying to grow cell lines from them. He also wanted to grow stem cells from mammalian embryos and study them in vitro. This began one of my most memorable 12 months of research. John’s laboratory had facilities unknown anywhere else, with CO2 incubators, numerous cell lines in constant cultivation, cryopreservation facilities, and the use of media droplets held under liquid paraffin. We decided to start with rabbits. Cell lines did not grow easily from cleaving rabbit embryos. In contrast, stem cells migrated out
in massive numbers from cultures of rabbit blastocysts, forming muscle, nerves, phagocytes, blood islands, and other tissues in vitro (12). Stem cells were differentiating in vitro into virtually all the tissues of the body. In contrast, dissecting the inner cell mass from blastocysts and culturing it intact or as disaggregated cells produced lines of cells that divided and divided, without ever differentiating. One line of these embryonic stem cells expressed specific enzymes, diploid chromosomes, and a fibroblastic structure as it grew over 200 and more generations. Another was epithelioid and had different enzymes but was similar in other respects. The ability to make whole-embryo cultures producing differentiating cells was now combined with everlasting lines of undifferentiated stem cells that replicated over many years without changing. Ideas of using stem cells for grafting to overcome organ damage in recipients began to emerge. My thoughts returned constantly to growing stem cells from human embryos to repair defects in tissues of children and adults.

Almost at my last moment in Glasgow, with this new set of ideas in my mind, a piece of excised ovary yielded several oocytes. Being placed in vitro, two of them had reached metaphase II and expelled a polar body at 37 hours. This showed that another target on the road to human IVF had been achieved as the whole pattern of oocyte maturation continued to emerge but with increasing clarity.

Cambridge University, my next and final habitation, is an astonishing place. Looking back on those days, it seems that the Physiological Laboratory was not the ideal place to settle in that august university. Nevertheless, a mixture of immunology and reproduction remained my dominant theme as I rejoined Alan Parkes and Bunny Austin there. I had to do immunology to obtain a grant to support my family, but thoughts of human oocytes and embryos were never far away. One possible model of the human situation was the cow and other agricultural species, and large numbers of cow, pig, and sheep oocytes were available from ovaries given to me by the local slaughterhouse. Each species had its own timing, all of them longer than 12 hours (13). Pig oocytes were closest to humans, requiring 37 hours. In each species, maturation timings in vitro were exactly the same as those arising in vivo in response to an hCG injection. This made me suspect that a woman would ovulate 36–37 hours after an injection of hCG. Human oocytes also trickled in, improving my provisional timings of maturation, and one or two of them were inseminated, but without signs of fertilization.

More oocytes were urgently needed to conclude the timings of oocyte meiosis. Surgeons in Johns Hopkins Hospital, Baltimore, performed the Stein–Leventhal operation, which would allow me to collect ovarian tissue, aspirate oocytes from their follicles, and retain the remaining ovarian tissues for pathology if necessary.

I had already met Victor McKusick, who worked in Johns Hopkins, at many conferences. I asked for his support for my request to work with the hospital gynecologists for six weeks. He found a source of funds, made laboratory space available, and gave me a wonderful invitation that introduced me to Howard and Georgeanna Jones. This significant moment was equal to my meeting with Molly Rose. The Joneses proved to be superb and unstinting in their support. Sufficient wedges and other ovarian fragments were available to complete my maturation program in human oocytes. Within three weeks, every stage of meiosis was classified and timed (14). We also undertook preliminary studies on inseminating human oocytes that had matured in vitro, trying to achieve sperm capacitation by using different media or adding fragments of ampulla to the cultures, and even attempting fertilization in rhesus monkey oviducts. Two nuclei were found in some inseminated eggs, resembling pronuclei, but sperm tails were not identified, so no claims could be made (15). During those six weeks, however, oocyte maturation was fully timed at 37 hours, permitting me now to predict with certainty that women would ovulate at 37 hours after an hCG injection.

A simple means of access to the human ovary was now essential in order to identify human ovarian follicles in vivo and to aspirate them 36 hours after hCG, just before the follicular rupture. Who could provide this? And how about sperm capacitation? Only in hamsters had fertilization in vitro been achieved, using in vivo-matured oocytes and epididymal spermatozoa (16). I met Victor Lewis, my third clinical colleague, and we noticed what seemed to be anaphase II in some inseminated eggs. Again, no sperm tails were seen within the eggs.

An attempt to achieve human capacitation in Chapel Hill, North Carolina, working with Robert McGaughey and his colleagues, also failed (17). A small intrauterine chamber lined with porous membrane was filled with washed human spermatozoa, sealed, and inserted overnight into the uterus of human volunteers at mid-cycle. Molecules entering it could react with the spermatozoa. No matured human eggs were fertilized. Later evidence indicated that the chamber contained inflammatory proteins, perhaps explaining the failure.

**DECISIVE STEPS TO CLINICAL HUMAN IVF**

Back in the United Kingdom, my intention to conceive human children in vitro had grown even stronger. So many medical advantages could flow from it. A small number of human embryos had been flushed from human oviducts or uteri after sexual intercourse, providing slender information on these earliest stages of human embryology. It was time to attain human fertilization in vitro, in order to move close to working with infertile patients. Ethical issues and moral decisions would emerge, one after the other, in full public view. Matters such as cloning and sexing embryos, the risk of abnormalities in the children, the clinical use of embryo stem cells, the ethics of oocyte donation and surrogate pregnancy, and the right to initiate human embryonic life in vitro would never be very far away. These issues were all acceptable, since I was confident that studies of human conception were essential for future medicine, and correct ethically, medically, and scientifically. The increasing knowledge of genetics and embryology could assist many patients if I could achieve
human fertilization and grow embryos for replacement into their mothers.

Few human oocytes were available in the United Kingdom. Despite this scarcity, one or two of those matured and fertilized in vitro possessed two nuclei after insemination. But there were no obvious sperm tails. I devised a cow model for human fertilization, using in vitro-matured oocytes and insemination in vitro with selected samples of highly active, washed bull spermatozoa extracted from neat semen. It was a pleasure to see some fertilized bovine eggs, with sperm tails and characteristic pronuclei, especially using spermatozoa from one particular bull. Here was a model for human IVF and a prelude to a series of events that implied that matters in my research were suddenly changing. A colleague had stressed that formalin fixatives were needed to detect sperm tails in eggs. Barry Bavister joined our team to study for his PhD and designed a medium of high pH, which gave excellent fertilization rates in hamsters. We decided to collaborate by using it for trials on human fertilization in vitro.

Finally, while browsing in the library of the Physiological Laboratory, I read a paper in The Lancet that instantly caught my attention. Written by Dr. P.C. Steptoe of the Oldham and District General Hospital (18), it described laparoscopy, with its narrow telescope and instruments and its minute abdominal incisions. He could visualize the ampulla and place small amounts of medium there, to gain working space in the abdominal cavity and used carbon fibers to pass cold light into the abdomen from an external source (25).

The beginnings of human in vitro fertilization

Patrick and I began our collaboration six months later in the Oldham and District General Hospital, almost 200 miles north of Cambridge. He had worked closely with two pioneers, Palmer in Paris (23) and Fragenheim in Germany (24). He improved the pneumoperitoneum to gain working space in the abdominal cavity and used carbon fibers to pass cold light into the abdomen from an external source (25). By now, Patrick was waiting in the wings, ready to begin clinical IVF in distant Oldham. We had a long talk about ethics and found our stances to be very similar.

Work started in the Oldham and District General Hospital and moved later to Kershaw’s Hospital, set up by my assistants, especially Jean Purdy. We knew the routine. It was based on my Edinburgh experiences with mice. Piero Donini from Serono Laboratories in Rome had purified urinary human menopausal gonadotropin (hMG) as a source of FSH and the product was used clinically to stimulate follicle growth in anovulatory women by Bruno Lunenfeld (26). It removed the need for PMS, thus avoiding the use of nonhuman hormones. We used low dosage levels in patients; that is, two to three vials (a total of 150–225 IU) given on days 3 and 5, and 5000–7000 IU of hCG on day 10. Initially, the timing of oocyte maturation in vitro was confirmed by performing laparoscopic collections of oocytes from ovarian follicles at 28 hours after hCG to check that they were in metaphase I (27). We then moved to 36 hours to aspirate mature metaphase II oocytes for fertilization. Those beautiful oocytes were surrounded by masses of viscous cumulus cells and were maturing exactly as predicted. We witnessed follicular rupture at 37 hours through the laparoscope. Follicles could be classified from their appearance as ovulatory or nonovulatory, this diagnosis being confirmed later by assaying several steroids in the aspirated follicular fluids (Figure 1.2).

It was a pleasure and a new duty to meet the patients searching for help to alleviate their infertility. We did our best, driving from Cambridge to Oldham and arriving at...
The beginnings of human *in vitro* fertilization

Patrick had stimulated the patients with hMG and hCG, and he and his team led by Muriel Harris arrived to prepare for surgery. Patrick’s laparoscopy was superb. Ovarian stimulation, even though mild, produced five or six mature follicles per patient, and ripe oocytes came in a steady stream into my culture medium for insemination and overnight incubation. The next morning, the formation of two pronuclei and sperm tails indicated fertilization had occurred, even in simple media, now with a near-neutral pH. Complex culture media, Ham’s F10 and others, each with added serum or serum albumin, sustained early and later cleavages (28), and even more fascinating was the gradual appearance of morulae and then light, translucent blastocysts ([Figure I.3](#)) (29). Here was my reward—growing embryos was now a routine, and examinations of many of them convinced me that the time had come to replace them into the mother’s uterus. I had become highly familiar with the teratologic principles of embryonic development, and knew many teratologists. The only worry I had was the chance of chromosomal monosomy or trisomy, on the basis of our mouse studies, but these conditions could be detected later in gestation by amniocentesis. Our human studies had surpassed work on all animals, a point that was highlighted even more when we grew blastocysts to day 9 after they had hatched from their zona pellucida ([Figure I.4](#)) (30). This beautifully expanded blastocyst had a large embryonic disc that was shouting that it was a potential source of embryonic stem cells.

When human blastocysts became available, we tried to sex them using the sex chromatin body as in rabbits. Unfortunately, they failed to express either sex chromatin or the male Y body so we were unable to sex them as female or male embryos. Human preimplantation genetic diagnosis would have to wait a little longer.

During these years there were very few plaudits for us, as many people spoke against IVF. Criticism was mostly aimed at me, as usual when scientists bring new challenges to society. Criticism came not only from the Pope and archbishops, but also from scientists who should have known better, including James Watson (who testified to a U.S. Senate Committee that many abnormal babies would

---

**Figure I.1** A composite picture of the stages of fertilization of the human egg. (*Upper left*) An egg with a first polar body and spermatozoa attached to the outer zona pellucida. (*Upper central*) Spermatozoa are migrating through the zona pellucida. (*Upper right*) A spermatozoon with a tail beating outside the zona pellucida is attaching to the oocyte vitelline membrane. (*Lower left*) A spermatozoon in the ooplasm, with enlarging head and distinct mid-piece and tail. (*Lower central*) Further development of the sperm head in the ooplasm. (*Lower right*) A pronucleate egg with two pronuclei and polar bodies. Notice that the pronuclei are apparently aligned with the polar bodies, although more dimensions must be scored to ensure that polarity has been established in all axes.
be born), and Max Perutz, who supported him. These scientist critics knew virtually nothing about my field, so who advised them to make such ridiculous charges? Cloning football teams or intelligentsia was always raised by ethicists, which clearly dominated their thoughts rather than the intense hopes of our infertile patients. Yet one theologian, Gordon Dunstan, who became a close friend, knew all about IVF from us, and wrote an excellent book on its ethics. He was far ahead of almost every scientist in my field of study. Our patients also gave us their staunch support, and so did the Oldham Ethical Committee, Bunny Austin back home in Cambridge, and Elliott Philip, a colleague of Patrick’s.

Growing embryos became a routine, so we decided to transfer one each to several patients. Here again we were in untested waters. Transferring embryos via the cervical canal, the obvious route to the uterus, was virtually a new and untested method. We would have to do our best. From now on, we worked with patients who had seriously distorted tubes or none whatsoever. This step was essential, since no one would have believed we had established a test-tube baby in a woman with near-normal tubes. This had to be a condition of our initial work. Curiously, it led many people to make the big mistake of believing that we started IVF to bypass occluded oviducts. Yet we already knew that embryos could be obtained for men with oligozoospermia or antibodies to their gametes, and for women in various stages of endometriosis.

One endocrinological problem did worry me. Stimulation with hMG and hCG shortened the succeeding luteal phase, leaving only a very short time for embryos to implant before the onset of menstruation. Levels of urinary pregnanediol also declined soon after oocyte collection. This condition was not a result of the aspiration of granulosa and cumulus cells, and luteal support would be needed, preferably progesterone. Csapo et al. stressed how this hormone was produced by the ovaries for the first 8–10 weeks before the placenta took over this function (31). Injections of progesterone in oil given over that long period of time seemed unacceptable since it would be extremely uncomfortable for patients. While mulling over this problem, my attention turned to those earlier endocrinologists who believed that exogenous hormones would distort the reproductive cycle, although I doubt they even knew anything about a deficient luteal phase.

This is how we unknowingly made our biggest mistake in the early IVF days. Our choice of Primolut® (Sigma Chemical Co., St. Louis, Missouri) depot, a progestogen, meant it should be given every five days to sustain pregnancies, since it was supposed to save threatened abortions. So, we began embryo transfers to patients in stimulated cycles, giving this luteal phase support. Even though our work was slowed down by having to wait to see whether pregnancies arose in one group of patients before stimulating the next, enough patients had accumulated after two to three years. None of our patients was pregnant, and

Figure I.2 Eight steroids were assayed in fluids extracted from human follicles aspirated 36–37 hours after the human chorionic gonadotropin (hCG) shot. The follicles had been classified as ovulating or non-ovulating by laparoscopic examination in vivo. Data were analyzed by cluster analysis, which groups follicles with similar features. The upper illustration shows data collected during the natural menstrual cycle. Note that two sharply separated groups of follicles were identified, each with very low levels of within-group variance. Attempting to combine the two groups resulted in a massive increase of within-group variation, indicating that two sharply different groups had been identified. These different groups accords exactly with the two groups identified by means of steroid assays. The lower figure shows the same analysis during stimulated cycles on fluids collected 36–37 hours after injecting hCG. With this form of stimulation, follicle growth displays considerable variation within groups. Attempts to combine all the groups result in a moderately large increase in variation. This evidence suggests that follicles vary considerably in their state of development in stimulated cycles using human menopausal gonadotropin (hMG) and hCG.
The beginnings of human in vitro fertilization

disaster loomed. Our critics were even more vociferous as the years passed, and the mutual support between Patrick and I had to pull us through.

Twenty or more different factors could have caused our failure; for example, cervical embryo transfers, abnormal embryos, toxic culture dishes or catheters, inadequate luteal support, incompatibility between patients’ cycles and that imposed by hMG and hCG, inherent weakness in human implantation, and many others. We had to glean every scrap of information from our failures. I knew Ken Bagshawe in London, who was working with improved assay methods for gonadotropic hormones. He offered to measure blood samples taken from our patients over the implantation period using his new hCG assay. He telephoned: three or more of our patients previously undiagnosed had actually produced short-lived rises of hCG over this period. Everything changed with this information. We had established pregnancies after all, but they had aborted very early. We called them biochemical pregnancies, a term that still remains today. It had taken us almost three years to identify the cause of our failure, and the finger of suspicion pointed straight at Primulot. I knew it was luteolytic, but it was apparently also an abortifacient, and our ethical decision to use it had caused much heartache, immense loss of work and time, and despair for some of our patients. The social pressures had been immense, with critics claiming our embryos were dud and our whole program was a waste of time; but we had come through it and now knew exactly what to do next.

We accordingly reduced the levels of Primulot depot, and utilized hCG and progesterone as luteal aids. Suspicions were also emerging that human embryos were very poor at implanting. We had replaced single embryos into most of our patients, rarely two. Increasingly we began to wonder whether more should be replaced, as when we replaced two in a program involving transfer of oocytes and spermatozoa into the ampulla so that fertilization could occur in vivo. This procedure was later called gamete intrafallopian transfer (GIFT) by Ricardo Asch. We now suspected that single embryo transfers could produce a 15%–20% chance of establishing pregnancy, just as our first clinical pregnancy arose after the transfer of a single blastocyst in a patient stimulated with hMG and hCG (32). Then came the fantastic news—a human embryo fertilized and grown in vitro had produced a pregnancy. Everything seemed fine, even with ultrasound images. My culture protocols

Figure I.3 Successive stages of human preimplantation development in vitro in a composite illustration made in Oldham in 1971. (Upper left) Four-cell stage showing the crossed blastomeres typical of most mammals. (Upper middle) Eight-cell stage showing the even outline of blastomeres and a small piece of cumulus adherent to the zona pellucida. (Upper right) A 16–32-cell stage showing the onset of compaction of the outer blastomeres. Often, blastocelic fluid can be seen accumulating between individual cells to give a “stripy” appearance to the embryo. (Lower left and middle) Two living blastocysts showing a distinct inner cell mass, single-celled trophoderm, blastocelic cavity, and thinning zona pellucida. (Lower right) A fixed preparation of a human blastocyst at five days, showing more than 100 even-sized nuclei and many mitoses.
were satisfactory after all. Patrick rang: he feared the pregnancy was ectopic and he had to remove it sometime after 10 gestational weeks. Every new approach we tested seemed to be ending in disaster, yet we would not stop, since the work itself seemed highly ethical, and conceiving a child for our patients was perhaps the most wonderful thing anyone could do for them. In any case, ectopic pregnancies are now known to be a regular feature with assisted conception.

I sensed that we were entering the final phase of our Oldham work, seven years after it began. We had to speed up, partly because Patrick was close to retiring from the National Health Service. Four stimulation protocols were tested in an attempt to avoid problems with the luteal phase: hMG and hCG; clomiphene, hMG, and hCG to gain a better luteal phase; bromocriptine, hMG, and hCG because some patients had high prolactin concentrations; and hCG alone at mid-cycle. We also tested what came to be known as GIFT, calling it oocyte recovery with tubal insemination (ORTI). In this treatment cycle, using human menopausal gonadotropin (hMG) and human chorionic gonadotropin (hCG), including additional injections of hCG for luteal support, a single preovulatory oocyte and 1.6 million sperm were transferred into the ampulla. Return to menstruation (RTM) indicates stages of the menstrual cycle. Abbreviations: LMP, last menstrual period; ODGH, Oldham and District General Hospital.

Lesley and John Brown came as the second entrants for natural-cycle IVF. Lesley had no oviducts. Her egg was aspirated in a few moments and inseminated simply and efficiently. The embryo grew beautifully and was transferred an hour or so after it became eight cells. Their positive pregnancy test a few days after transfer was another milestone—surely nothing could now prevent their embryo developing to full term in a normal reproductive cycle, but those nine months lasted a very long time. Three more pregnancies were established using natural-cycle IVF as we abandoned the other approaches. A triploid embryo died in utero—more bad luck. A third pregnancy was lost through premature labor on a mountain walking holiday, two weeks after the mother’s amniocentesis (32,33). It was a lovely, well-developed boy. Louise Brown’s birth, and then Alistair’s, proved to a waiting world that science and medicine had entered human conception. Our critics declared that the births were a fake, and advised against attending our presentation on the whole of the Oldham work at the Royal College of Obstetricians and Gynaecologists.

### IVF WORLDWIDE

The Oldham period was over. Good facilities were now needed, with space for a large IVF clinic. Bourn Hall was an old Jacobean house in lovely grounds near Cambridge (Figure I.7). The facilities on offer for IVF in Cambridge were far too small, so we purchased it mostly with venture capital. It was essential to conceive 100 or 1000 IVF babies
The beginnings of human *in vitro* fertilization to ensure that the method was safe and effective clinically. The immense delays in establishing Bourn Hall delayed our work by two years after Louise’s birth. Finally, on minimal finance, Bourn Hall was opened in September 1980 on a shoestring, supported by our own cash and loans. The delay gave the rest of the world a chance to join in IVF. Alex Lopata delivered an IVF baby in Australia, and one or two others were born elsewhere. Natural-cycle IVF was chosen initially at Bourn Hall since it had proved successful in Oldham, and we became experts in it. Pregnancies flowed, at 15% per cycle. An Australian team of Alan Trounson and Carl Wood announced the establishment of several IVF pregnancies after stimulation by clomiphene and hCG and replacing two or three embryos (34), so they had moved ahead of us.

**Figure I.6** Recording the progress of the human natural menstrual cycle for *in vitro* fertilization (IVF). Three patients are illustrated. All three displayed rising 24-hour urinary estrogen concentrations during the follicular phase and rising urinary pregnanediol concentrations in the luteal phase. Luteinizing hormone (LH) levels were measured several times daily and the data clearly reveal the exact time of onset of the LH surge.

**Figure I.7** Bourn Hall (courtesy of Dr. P Brinsden).
during the delayed opening of Bourn Hall. Our own effort now expanded prodigiously: Thousands of patients queued for IVF. Simon Fishel, Jacques Cohen, and Carol Fehilly joined the embryology team among younger trainees, and new clinicians joined Patrick and John Webster. Patients and pregnancies increased rapidly, and the world was left standing far behind. Howard and Georgieanna Jones began in Norfolk using gonadotropins for ovarian stimulation. Jean Cohen began in Paris, Wilfred Feichtinger and Peter Kemeter in Vienna, Klaus Diedrich and Hans van der Venn in Bonn, Lars Hamberger and Matts Wikland in Sweden, and Andre van Steirteghem and Paul Devroey in Brussels. IVF was now truly international.

The opening of Bourn Hall had not deterred our critics. They put up a fierce rearguard action against IVF, alongside LIFE, Society for the Unborn Child, individual gynecologists, and others.

Objections raised against IVF included low rates of pregnancy (no one mentioned the similar low rates of pregnancy with natural conception), the possibilities of oocyte and embryo donation, surrogate mothers, unmarried parents, one-sex parents, embryo cryopreservation, cloning, and endless other objections.

LIFE issued a legal action against me for the abortion of an embryo grown for 14 days and longer in vitro. Their action was rejected by the U.K. Attorney General since the laws of pregnancy began after implantation. We fully respected the intense ethical nature of our proceedings. We also recognized the need for research, and the necessity to protect or cryopreserve the best embryos for later replacement into their mothers. Those not replaced had to be used for research under strict controls, combined with open publication and discussion of our work.

Each year, 1000—rising to almost 2000—patients passed through Bourn Hall. Different stimulation regimens or new procedures could be tested in very little time. Clomiphene/hMG was reintroduced. Bourn babies increased: 20, 50, 100–1000 after five to six years. This was far more than half of the world’s entire IVF babies, including the first born in the U.S.A., Germany, Italy, and many other countries. Detailed studies were performed on embryo culture, implantation, and abortion. We even tried aspirating epididymal spermatozoa for IVF, without achieving successful fertilization.

Among the immense numbers of patients, people with astonishingly varied conditions of infertility emerged. Some were poor responders in whom immense amounts of endocrine priming were essential, some were women with a normal menstrual cycle that was not as it should have been, some had previous misdiagnoses that had laid the cause of infertility on the wife when the husband had never even been investigated, and some were men bringing semen samples that we discovered had been obtained from a friend. The collaboration between nurses, clinicians, and scientists was remarkable. Yet trouble—ethical trouble—was never far away. I purchased a freezing machine to resume our Oldham work, but, unknown to me, Patrick talked to officers of the British Medical Association (BMA) and for some reason agreed to delay embryo cryopreservation. Apparently, the BMA felt it would be an unwelcome social development. I did not approve of these reservations: David Whittingham had shown how low-temperature cryostorage was successful with mouse embryos, without causing genetic damage. “Freezing and cloning” became a term of intense approbation at this time. I unwillingly curtailed our cryopreservation program.

One weekend, major trouble erupted as a result of this difference between Patrick and me. My duties in Bourn Hall prevented me from attending a conference in London. Trying to be helpful, I telephoned my lecture to London. Reception at the other end was apparently so poor as to lead to misinterpretations of what I had said. Next morning, the press furor about my supposed practice of cryopreserving embryos after IVF was awful; so bad, indeed, that legal action had to be taken. Luckily, my lecture had been recorded, and listening to the tapes with a barrister revealed nothing contentious. I had said nothing improper in my lecture or during the question-and-answer session. That day, I issued seven libel actions against the cream of British society: the BMA and its secretary, the BBC, The Times, and other leading newspapers. There were seven in one day and another one later! If only one was lost, I could be ruined and disgraced. However, they were all won, even though it took several years with the BMA and its secretary. These legal actions had inhibited our research, with the cryopreservation program being shut down for more than a year. Every single embryological note of mine from those days in Oldham and from Bourn Hall was examined in detail for my opponents by someone who was clearly an embryologist. Nothing was found to incriminate me.

That wretched period passed. The number of babies kept on growing, embryo cryopreservation was resumed, and Gerhard Zeilmaker in the Netherlands beat us and the world to the first “ice” baby (35). Colin Howles and Mike McNamee joined us in endocrinology and Mike Ashwood-Smith and Peter Holland’s joined us in embryology as the old team faded away. Fascinating days had returned. Working with barristers, we designed consent forms that were far in advance of those used elsewhere. Oocyte donation and surrogacy by embryo transfer were introduced. The world’s first paper on embryo stem cells appeared in Science in 1984, sent from Bourn Hall, and the world’s first paper on human preimplantation diagnosis in 1987 appeared in Human Reproduction. However, embryo research faltered as all normal embryos were cryopreserved for their parents, so almost none were available for study. Alan Handyside, one of our Cambridge PhDs, joined Hammersmith Hospital in London to make major steps in introducing preimplantation genetic diagnosis (36). As we reached 1000 pregnancies, our data showed the babies to be as normal as those conceived in vivo.

Test-tube babies (an awful term) were no longer unique and were accepted worldwide, exactly as Patrick and I had hoped. Our work was being recognized (Figure 1.8). Clinics sprang up everywhere. Ultrasound was introduced to detect follicles for aspiration by the Scandinavians (37),
making laparoscopy for oocyte recovery largely redundant. Artificial cycles were introduced in Australia and intracytoplasmic sperm injection (ICSI) was introduced in Belgium (38), and gonadotropin-releasing hormone agonists were used to inhibit the LH surge. Ian Craft in London showed how postmenopausal women aged 52 or more could establish pregnancies using oocyte donation and endocrine support. Women over 60 years of age conceived and delivered children. This breakthrough was especially welcome to me, since older women surely have the right to have children at ages almost the same as those possible for men.

Ethics continued side by side with advancing science and medicine. The U.K. governmental Warnock report recommended permitting embryo research and proposed a Licensing Authority for IVF. A year or so later, the U.K. House of Lords, in all its finery, responded with a 3:1 vote in favor, decisive support for all we had done in Mill Hill, Cambridge, and Oldham. What a wonderful day! The British House of Commons passed a liberal IVF law after intense debate, and so did the Spanish government, although elsewhere things were not so liberal. Ten years after the birth of Louise Brown, the British Parliament had therefore accepted IVF, research on human embryos until day 14, and establishing research embryos. Cloning and embryo stem cells still bothered the politicians of 1988, only to re-emerge in 1998, gray shadows of my earlier times in Glasgow. IVF had also become fundamental to culture embryos to cause, for example, large-calf syndrome. It would be wise to be well aware of these findings when practicing human IVF; for example, by assessing the role of sera in human culture media.

**IVF OUTLOOK**

In one sense, opening up human conception in vitro was perhaps among the first examples of applied science in modern “hi tech.” Human IVF has since spread throughout the world, with apparently more than 3.5 million such babies born worldwide by 2008—yet Louise Brown is only just 30 years of age. The need for IVF and its derivatives is greater than ever, since up to 10% of couples may suffer from some form of infertility. Major advances in genetic technologies now identify hundreds of genes in a single cell, and diagnosing genetic disease in embryos promises to help avoid desperate genetic diseases in newborn children. Indeed, the ethics of this field have now become even more serious, since the typing of embryo genotypes provides detailed predictions of future life and health.

IVF has now combined closely with genetics to eliminate disease or disability genes, or lengthen the life span.
But most of all, practicing IVF teaches a wider understanding of the desire and love for a child and a partner, the wonderful and ancient joys of parenthood, the pain of failure, and the deep motivation needed in donating and receiving an urgently needed oocyte or a surrogate uterus. Parenthood is more responsible than ever before. Its complex choices are gathered before couples everywhere by the information revolution, placing family responsibilities on patients themselves, where it really matters. And IVF now reveals more and more about miracles preserved in embryogenesis from flies and frogs to humankind, over 600 million years of evolution.

The Human Genome Project is now complete and will inevitably assist IVF since we will soon understand the genetic aspects of early embryo growth and how to detect abnormalities preserved in embryos from flies and frogs to humankind, over 600 million years of evolution.

REFERENCES

Robert G. Edwards and the thorny path to the birth of Louise Brown: A history of in vitro fertilization and embryo transfer

MARTIN H. JOHNSON MA, PHD, FRCOG, FRSB, FMEDSCI, FRS

INTRODUCTION

Robert G. Edwards was awarded the 2010 Nobel Prize for Physiology or Medicine “for the development of in vitro fertilization” (1). There is a variety of accounts of the events leading up to this discovery and its acceptance, most of them by participants (2), but historical scholarship is rarer (3). This article is based on research undertaken partly in preparation for the introductory lecture to the Nobel Symposium celebrating the award of the 2010 Nobel Prize in Physiology or Medicine to Robert G. Edwards, and partly conducted since then. It is based on a paper published originally in 2011 (1), but adds considerably to that paper by use of verifiable sources to produce a historical narrative of the path to in vitro fertilization (IVF) and the birth of Louise Brown that differs in a number of places from the conventionally accepted version and adds further detail. It tries to make clear what a difficult birth IVF had, something often overlooked by current practitioners.

Primary sources used were the publications by Edwards and Steptoe between the 1950s and 1980s; the National Archives, the archives of the Royal Society of Medicine, Cambridge University, the British Medical Association, Churchill College, Cambridge, the Physiology Library at Cambridge, the National Institute for Medical Research (NIMR) at Mill Hill and the personal papers of Robert G. Edwards (courtesy of the Edwards family); transcripts of interviews with Robert G. Edwards (unpublished), with K. Elder and R.L. Gardner (available from the British Library Oral History Section), and with Grace MacDonald (4), Noni Fallows, Sandra Corbertt, and John Webster (5); personal recollections from the late 1970s by Edwards and Steptoe as recalled in interviews with Danny Abse for the autobiographical account A Matter of Life and on film with Peter Williams; and members of Robert G. Edwards’ family and his colleagues and former students and staff members for clarificatory evidence about personal recollections by Edwards, for additional verifiable information and with whom to test some new interpretations.

CHILDHOOD BACKGROUND

Robert G. Edwards was born on September 27, 1925 into a working-class family in the small Yorkshire mill town of Batley. Edwards, who was known by his middle name of Geoff until he was 18, was the second of three brothers, between an older brother, Sammy, and the younger, Harry (2). Sammy was named after his father, Samuel, who was frequently away from home working on the railways, maintaining the track in the Blea Moor tunnel on the Carlisle–Settle line. It was an unhealthy place to work, filled with coal-fired smoke that exacerbated Samuel’s bronchitis, a consequence of being gassed in World War I. Edwards’ mother, Margaret, was a machinist in a local mill. She came originally from Manchester, to where the family relocated when Edwards was about five, having been offered the relative security of a council house in the suburb of Gorton. It was in Manchester that Edwards received his education: bright working-class children could take a scholarship exam at age 10 or 11 to compete for the few coveted places at a grammar school, the potential pathway out of poverty and even to university. All three brothers passed the exam, but Sammy decided against grammar school, preferring to leave education as soon as he could to earn. His mother was reportedly furious at this wasted opportunity, and so when her two younger sons passed the exam, there was no doubting that they would continue in education, and so it was that that Geoff/Bob progressed in 1937 to Manchester Central Boy’s High School, which also claims James Chadwick FRS (1891–1974) as an alumnus. Chadwick, like Edwards, became a Cambridge professor and was awarded the 1935 Nobel Prize in Physics for discovering the neutron (6). The Edwards family’s summers were spent in the Yorkshire Dales, to where their mother took her sons to be closer to their father’s place of work. There, Edwards labored on the farms and developed an enduring affection for the Dales. These early experiences were formative for Edwards in three ways. Thus, Edwards became a life-long egalitarian, for five years a Labour Party councilor and almost selected as the Labour parliamentary candidate for Cambridge (7), willing to listen and talk with all and sundry, regardless of class, education, status, and background. Second he also developed an enduring curiosity about agricultural and natural history and especially the reproductive patterns among the Dales’ sheep, pigs, and cattle. Finally, he claimed great pride in being a “Yorkshire man,” traditionally having attributes of affability and generosity of spirit combined with no-nonsense blunt speaking. Indeed, following his only meeting with Gregory Pincus (1903–1967 [8]) at a conference in Venice in May 1966, at which Edwards, the young pretender, clashed with the “father of the pill” over the timing of egg maturation in humans, he paid
Pincus the biggest compliment he could then imagine, saying, “He would have made a fine Yorkshireman!” (2).

The intervention of World War II provided an unwelcome interruption to Edwards’ education, for, after leaving school in 1943, he was conscripted for war service into the British Army for almost four years (Figure II.1). To his surprise, as someone from a working-class family, he was identified as potential officer material and sent on an officer-training course, before being commissioned in 1946. However, his army experiences were broadly negative, the alien lifestyle of the officers’ mess reinforcing his socialist ideals. The one positive feature of his war service was the chance to travel overseas; he particularly appreciated his time in the Middle East. The years in the army were broken by nine months’ compassionate leave back in the Yorkshire Dales, for which he was released to help run a farm when his farmer friend there fell ill. So engaged did he become in farming life that, after discharge from the army in 1948, he returned home to Gorton, from where he applied to read agricultural sciences at the University College of North Wales at Bangor and gained both a place and a government grant to fund it. However, he was disappointed in the course offered at Bangor, describing it as not “scientific,” and he was bored through two tedious years of agricultural descriptions. For his third year, he transferred to zoology, a course much more to his style and led by the more intellectually challenging Rogers Brambell FRS (1901–1970 [9]), the first Professor of Reproductive Sciences at Cambridge, who was later to recruit Edwards there, distinguished themselves as undergraduates. In 1951, however, Edwards “was disconsolate. It was a disaster. My grants were spent and I was in debt. Unlike some of the students I had no rich parents … I could not write home, ‘Dear Dad, please send me £100 as I did badly in the exams’” (2).

However, his low spirits did not last long. He learnt that John Slee (12), a life-long friend he had made at Bangor, had been accepted on a postgraduate diploma course in animal genetics at Edinburgh University under Conrad Waddington FRS (1905–1975 [13]), who had moved there in 1947 from Christ’s College in Cambridge, home also to both Marshall and Parkes. Edwards applied and, despite his pass degree and to his amazement, he was accepted. That summer, he worked harvesting hay, portering bananas, heaving sacks of flour, and in a menial job with a newspaper, all to earn enough to pay his way in Edinburgh (2).

**FAMILY LIFE**

In Edinburgh, Edwards not only started to map out his scientific career, but importantly also met Ruth Fowler (Figure II.2), who was to become his life-long scientific collaborator and whom he was to marry in 1954, with their five daughters arriving between 1959 and 1964. When they met, Edwards claims that he was initially somewhat overwhelmed, even “intimidated” by Ruth’s august family background. Her father, Sir Ralph Fowler FRS (1889–1944 [14]), and her maternal grandfather, Lord Ernest Rutherford FRS (1871–1937 [15]), were not only both “titled,” but both also had the most impressive academic credentials imaginable: a world away from a working-class Northern family. Ralph Fowler was an exceptionally talented Plummer Professor of Mathematical Physics in Cambridge from 1932 to 1944 (14). Back in Cambridge in 1919 after World War I, he was stimulated to work with Rutherford, who had recently arrived there to take the chair of Experimental Physics. Rutherford was the first Nobel laureate in Ruth’s family, having been awarded the 1908 Nobel Prize for Chemistry “for his investigations...
into the disintegration of the elements, and the chemistry of radioactive substances” (15). Ralph Fowler not only worked under Rutherford, but, in the course of doing so, met his only daughter, Eileen, whom he married in 1921. They had four children, of whom Ruth was the last, born in December 1930. Tragically her mother died shortly afterwards and her father, although himself unwell, undertook such grueling high-security war work, much of it away in North America, that his health deteriorated and he died at the relatively young age of 55 when Ruth was 13. Thus, Ruth was to know only Mrs. Phyllida Cook as her parent (14).

**EDWARDS, THE RESEARCH SCIENTIST**

The intellectual spirit of scientific enquiry that Edwards experienced in Edinburgh fitted his aptitudes well, for Waddington rewarded his Diploma year with a three-year PhD place (1952–1955), followed by two years of postdoctoral research, and funded it with a salary of the princely sum of £240 per year (2). His chosen field of research was the developmental biology of the mouse. Edwards realized that to understand development involved engaging in an interdisciplinary mix, not just of embryology and reproduction—the conventional view at the time—but also of genetics. Given the increasing scientific and social emphasis on genetics over the last 50 years, it is important to understand how advanced this view was in the 1950s, when genetic knowledge was still rudimentary and largely alien to the established developmental and reproductive biologists of the day, as Edwards himself was later to recall (16). For example, it was in the 1950s that DNA was established as the molecular carrier of genetic information (17–20), that it was first revealed that each cell of the body carried a full set of DNA genes (21–23), and that genes were selectively expressed as mRNA to generate different cell phenotypes (24). Moreover, it was only by the late 1950s that cytogenetic studies led to the accepted human karyotype as 46 chromosomes (25,26), that agreement was reached on the Denver system of classification of human chromosomes (27), and that the chromosomal aneuploidies underlying developmental anomalies such as Down, Turner, and Klinefelter syndromes were described (28–31). The dates of these discoveries make Edwards’ research between 1952 and 1957 all the more remarkable. Working under his supervisor Alan Beatty, he generated haploid, triploid, and aneuploid mouse embryos and studied their potential for development. In order to undertake what were, in effect, early attempts at “genetic engineering” in mammals, he needed to be able to manipulate the chromosomal composition of eggs, spermatozoa, and embryos.

In mice, spermatozoa were abundant, and were studied in experiments mostly undertaken with a visiting Argentinean postdoc, Julio Sirlin (Figure II.3) (2). Together they labeled spermatozoa radioactively in vivo in order to study the kinetics of spermatogenesis and then to follow the radioactive products post-fertilization, thereby to demonstrate the fate of the male contributions during early development. They also exposed males and/or their spermatozoa to various chemical mutagens and UV or x-ray irradiation, and examined the effects on sperm-fertilizing capacity and, where it was shown to be present, how the treatments impacted on development. In some cases, sperm activation of the egg was evident, but in the absence of any functional sperm chromatin, and so gynogenetic embryos were formed. These experiments resulted in 14 papers, including four in *Nature*, between 1954 and 1959 (see Gardner and Johnson [32] for a full bibliographic record of Edwards).

Eggs and embryos were not as abundant as spermatozoa, and overcoming this problem led Edwards to two discoveries that proved to be of particular significance for his later IVF work. First, working with his wife Ruth, he devised ways of increasing the numbers of synchronized eggs recoverable from adult female mice through a series of papers, the first published in 1957 (33), on the control of ovulation induced by use of exogenous hormones. In doing so, they overturned the conventional wisdom that superovulation of adults was not possible. Second, working with an American postdoc, Alan Gates (34), Edwards described the remarkable timed sequence of egg chromosomal maturation events that led up to ovulation after injection of the ovulatory hormone, human chorionic gonadotropin.

His six years in Edinburgh, between 1951 and 1957, give an early taste of his prodigious energy, resulting in 38 papers (32). Indeed, so productive was this period that the last of the Edinburgh-based papers did not appear in print until 1963. These papers firmly placed the young Edwards at the forefront of studies on the genetic manipulation of development and started to attract attention. It was also in Edinburgh that Edwards’ interest in ethics was first sparked by the interdisciplinary debates among scientists and theologians that Waddington organized, and, as a result, he went on what he describes as a “church crawl,” trying the 10 or so variants of Christianity on offer in 1950s Edinburgh. He did not emerge from his consumer testing “God-intoxicated” (2), but convinced that man held his own future in his own hands. Edwards’ humanist ethical sympathies and antipathy to the “revealed truths”
of religion were to be developed further in all his later encounters (32).

AN AMERICAN DIVERSION

These 1950s studies in science and ethics were to form the platform on which Edwards’ later IVF work was to be based, but before that his interests and life took a diversion to the California Institute of Technology for the year 1957–1958. He describes his year at Caltech as being “a bit of a holiday,” but it was a holiday that with hindsight had both distracting and significant consequences. He went there to work with Albert Tyler (1906–1968 [35]), an influential elder statesman of American reproductive science, working on spermatozoon–egg interactions. Caltech was then a hotbed of developmental biology, and Tyler had clustered around him an exciting group of young scientists, which included that year a visit by the then English doyen of fertilization, Lord Victor Rothschild FRS (1910–1990 [36]). Rothschild was later to clash scientifically with Edwards over his IVF work (37), a clash in which the younger man triumphed again (38), just as he had with Pincus. Tyler was exploring the molecular specificity of egg–spermatozoon interactions and had turned for a model to immunology. Immunology was then at an exciting phase in its development, with the engaging Sir Peter Medawar FRS (1915–1987, Nobel laureate in Physiology or Medicine, 1960 [39]), influentially for Bob, extending his ideas on immunological tolerance to the paradox of the “fetus as an allograft”: a semi-paternal graft nonetheless somehow protected from maternal immune attack inside the mother’s uterus (40). This confluence of reproduction and immunology excited Edwards’ restless curiosity and hence the choice of Tyler. Significantly, the subject also offered funding possibilities via the Ford and Rockefeller Foundations and the Population Council, which were increasingly concerned about world population growth and the need for better methods to control fertility (41–43). Immuno-contraception then seemed to offer tantalizingly specific possibilities, alas not much closer to being realized today (44).

So when Edwards returned to the United Kingdom from Caltech in 1958 at Alan Parkes’ invitation to join him at the Medical Research Council (MRC) National Institute for Medical Research (NIMR) in north London, it was to work on the science of immuno-contraception (7). This period in the U.S.A. initiated a series of 23 papers on the immunology of reproduction between 1960 and 1976 (32). It also prompted Edwards’ first involvement in founding an international society in 1967 in Varna, Bulgaria, when the International Coordinating Committee for the Immunology of Reproduction was created (45). Immuno-reproduction was, in retrospect, to prove a distracting diversion from what was to become Edwards’ main work, albeit one that continued to enthuse and stimulate his imagination for many years. Indeed, it was his research into immuno-reproduction that led serendipitously to his first meeting with Patrick Steptoe (see later). The period at Mill Hill, between 1958 and 1962, seems to have been a period of increasing intellectual conflict for him. While being enthusiastic about the science underlying immuno-contraception, his old interests in eggs, fertilization, and, in particular, the genetics of development were gradually reasserting themselves. His day job was therefore increasingly supplemented by evening and weekend flirtations with egg maturation.

THE CRUCIAL EGG MATURATION STUDIES

The stimulus that reawakened Edwards’ interest in eggs was provided by the then recent consensus about the number of human chromosomes and, more particularly, the descriptions in 1959 of the pathologies in man that resulted from chromosomal anomalies (28–31). Thus, his 1962 Nature paper begins: “Many of the chromosomal anomalies in man and animals arise through non-disjunction or lagging chromosomes during meiosis in the oocyte. Investigation of the origin and primary incidence of such anomalies would be greatly facilitated if meiotic stages etc., were easily available” (46). The idea that these aneuploidies in humans might result from errors in the complex chromosomal dance that he and Gates had observed in maturing mouse eggs drove his thinking. The possible clinical relevance of his work on egg maturation and aneuploidy in the mouse was becoming significant.

So Edwards resumed his experimenting with mice, trying to mimic in vitro the in vivo maturation of eggs, one rationale being that this route would open the possibility of similar studies in humans, in which not even induced ovulation had then been described (47). He tried releasing the immature mouse eggs from their ovarian follicles into culture medium containing the ovulatory hormone human chorionic gonadotropin, to explore whether he could simulate their in vivo development. Amazingly, he found it worked first time; the eggs seemed to mature at the same rate as they had in vivo. However, they did so whether or not the hormone had been added. The eggs evidently were maturing spontaneously when released from their follicles. The same happened in rats and hamsters. If this were to happen in humans too, then the study of the chromosomal dance during human egg maturation was a realistic practical possibility, as was IVF and thereby studies on the genetics of early human development. Edwards’ excitement at seeing eggs mature spontaneously was temporarily blunted by his library discovery that Pincus in the 1930s (48,49) and M.C. Chang (1908–1991 [50,51]) earlier in the 1950s had been there before him, using both rabbit and, Pincus claimed, human eggs.

In order to pursue his cytogenetic studies on the maturation of human eggs, he needed a reliable supply of human ovarian tissue from which to retrieve and mature eggs. This requirement posed difficulties for a scientist with no medical qualification, given the elitist attitudes and lack of scientific awareness then prevalent amongst most of the U.K. gynaecological profession (3,52,53). His first breakthrough came with Molly Rose, who was a gynaecologist at the Edgware General Hospital, northwest London, near Mill Hill. Edwards was introduced to her through John
Humphrey FR (1915–1997 [54]), who was the medically qualified Head of Immunology at Mill Hill. Humphrey, notwithstanding his more privileged social background, was a kindred spirit for Edwards, sharing his passion for science, its social application and utility, as well as his left-wing politics; indeed, he had been a Marxist until 1940 and was for many years denied entry to the U.S.A. in consequence. Edwards asked Humphrey if he knew anyone who might be helpful, and he not only suggested Rose, but also offered to arrange an introduction. Rose was to provide biopsied ovarian samples intermittently for the next 10 years.

Between 1960 and 1962, Edwards used human ovarian biopsies provided by Rose to try to repeat and extend Pincus’ observations from the 1930s. Given the sporadic supply of human material, he also tried dog, monkey, and baboon ovarian eggs, but in all cases with limited success compared with smaller rodents. In the 1962 Nature paper (46), he cautiously interprets the few maturing human (3/67), monkey (10/56), and baboon (13/90) eggs that he had observed as most likely arising from in vivo stimulation, rendering them partially matured at the time of their recovery from the biopsy. He suggests that Pincus’ observations on human eggs are also likely to be similarly artefactual, the source of his Venice spat with Pincus some four years later (vide supra). This 1962 paper ends with the report of an ingenious experimental approach to try and persuade the reluctant human eggs to mature. Thus, the ovarian arteries of patients undergoing ovarian removal were cannulated and perfused with hormones post-removal, perhaps unsurprisingly in retrospect, without success.

However, by this time, his quest for human eggs, and his dreams of IVF and studying the genetics and development of early human embryos, had reached the ears of the then Director of the Institute, Sir Charles Harington FR (1897–1972 [55]), who, Edwards alleged (2), banned any work on human IVF at NIMR. Alan Parkes was no longer able to defend Edwards, having left in 1961 to take up his chair in Cambridge and, although he had asked Edwards to join him there, funding was not available until 1963. So by the time Edwards left Mill Hill in 1962 for a year in Glasgow, he had encountered a taste of the opposition to human IVF that was to come.

GLASGOW AND STEM CELLS

Edwards had accepted an invitation from John Paul to spend a year in the biochemistry department at Glasgow University. Paul was then the acknowledged master of tissue culture in the U.K. and had got wind of some experiments that Edwards had been doing at NIMR attempting to generate stem cells from rabbit embryo cultures (56,57). The objective of this strategy was to use these stem cells to study early developmental mechanisms, either in vitro or in vivo after their incorporation into embryos. Paul had proposed that they work together, with fellow Glasgow biochemist Robin Cole, to see what progress might be made. This must have been an attractive invitation, not simply because the challenge was scientifically interesting, but also because Edwards could learn more about culture media for his eggs and hopefully later embryos, then an uncertain prospect, with successful mouse embryo culture only recently having been described (58). However, by this time, the Edwards family was growing, so Ruth remained in north London with their young daughters, while her husband commuted to Glasgow for the working week.

The collaboration was to result in two papers (56,57), remarkable for their prescience. They described the production of embryonic stem cells from both rabbit blastocysts and the inner cell masses dissected from them. The cells were capable of proliferating through over 100 generations and of differentiating into various cell types. These experiments were initiated some 20 years before Evans and Kaufman (59) described the derivation of embryonic stem cells from mice. That this work has largely been ignored by those in the stem cell field is probably mainly attributable to its being too far ahead of its time (60). Thus, reliable molecular markers for different types of cells were not available then, nor were appropriate techniques with which to critically test the developmental potential of the cultured cells.

THE MOVE TO CAMBRIDGE

Edwards arrived in Cambridge from Glasgow in 1963 as a Ford Foundation Research Fellow, and settled with Ruth and his five daughters in a house in Gough Way, off the Barton Road. He had previously visited Cambridge at least once, as “a recently graduated PhD” in the late 1950s for a conference on reproduction held in Trinity College (Figure II.4), where he recalls meeting some of the big names in the subject, including John Hammond, Alan Parkes, M.C. Chang, Thaddeus Mann, Rene Moricard, Bunny Austin, and Charles Thibault (16). Although Edwards was to remain in Cambridge for the rest of his career, in 1963 his initial reactions to the place were mixed. He describes how he immediately reacted against the then extant “misogynist public-school traditions; the exclusivity,” “the privileges given to the already privileged.” But he set against that the “sheer beauty of the place,” “the concern with the truth and high seriousness,” “the ambience of scientific excellence … I was surrounded by so many talented young men and women” (2).

Edwards worked in a cluster of seven smallish rooms at the top of the Physiological Laboratory backing onto Downing Place, which were collectively known as the “Marshall laboratory” and were to be shared eventually with two other groups. One group was led initially by Sir Alan Parkes, the first Mary Marshall, and Arthur Walton, Professor of Reproductive Physiology at the University (11), who had arrived in 1961. His group included scientists with mainly zoological or comparative interests, such as his wife Ruth Deansley, Bunny Austin, and Dick Laws FRS, who was often away “in the field” with Parkes collecting material, especially in Uganda at the Nuffield Unit of Tropical Animal Ecology (11). Parkes was also much involved at this time in writing and committee work,
especially with the World Health Organization, which was then becoming concerned about world population growth and ways to curb it (11). Parkes was also acting as an unpaid company secretary to the then fledgling Journal of Reproduction and Fertility (called Reproduction since 2001 [61–63]). In 1967, Parkes retired. Edwards applied for his chair on January 6, 1966 (64), but was unsuccessful, the chair passing instead to Thaddeus Mann FRS (1908–1993 [65]), who worked on the biochemistry of semen. Mann decided not to relocate to the Physiology Laboratory from his Cambridge base at the Agricultural Research Council Unit of Reproductive Physiology and Biochemistry at Huntingdon Road, where he was Director. Neither was the leadership of the Marshall laboratory to pass to Edwards, as the University appointed as its head his more senior colleague and friend Colin “Bunny” Austin (1914–2004 [66]), who had been in Cambridge intermittently since 1962 (Figure II.5). Austin was elected the first Charles Darwin Professor of Animal Embryology (1967–1981) and began attracting several upcoming reproductive biologists to the Marshall laboratory, including John Marston, David Whittingham, and Matthew Kaufmann. In addition, a new group was formed in 1967 with the arrival from the Strangeways laboratory of Denis New (1929–2010) as university lecturer in histology (67). New built a group comprising initially PhD students Chris Steele and David Cockroft, later joined by postdoc Frank Webb (1976–1977), and visiting scientists such as Joe Daniels Jr, on leave from the University of Colorado.

It was against this varied scientific background that Edwards, who was already 38 when he arrived in Cambridge, began for the first time to assemble his own group. He recruited as his technician Jean Purdy (Figure II.6) in 1968, one of her attractions being her nursing qualification, a sign of the increasing importance that his forays into the use of clinical material was assuming. Purdy was to stay with him until her early death at age 39 in 1985 (68). He also recruited his first two graduate students: Richard Gardner and this author in 1966 (69,70). Gardner studied early mouse embryology from 1966 to 1971 and until 1973 as a postdoctoral worker, before moving to zoology in Oxford. This author worked on immunoreproduction from 1966 to 1969, returning as a postdoc between 1971 and 1974 after two years in the U.S.A., before moving to the Anatomy Department in Cambridge.

From 1969 onwards, Edwards’ group increased in size substantially as more accommodation was made available...
Robert G. Edwards and the thorny path to the birth of Louise Brown: A history of in vitro fertilization and embryo transfer

xxxi

to the Marshall laboratory. David Griffin (now retired from the World Health Organization) was to join as Head Technician between 1970 and 1975, with junior technicians including Sheila Barton (1936–2013) in addition to Jean Purdy. Early graduate students recruited included Roger Gosden (1970–1974), Carol Readhead (1972–1976), and Rob Gore-Langton (1973–1978), all working on follicle growth; Craig Howe (1971–1974) working on immuno-reproduction; and Azim Surani (1975–1979) working on implantation. A “third generation” of graduate students also arrived; for example, Janet Rossant (from 1972) studied with Gardner, and Alan Handyside (from 1974), Peter Braude (from 1975), and Ginny Bolton (from 1976) studied with Johnson. Postdoctoral workers also arrived, including Ginny Papaioannou (1971–1974), and Ruth Fowler-Edwards resumed working in the laboratory, developing hormonal assays and studying the endocrine aspects of follicle development and early pregnancy. Thus, slowly until 1969, and more rapidly thereafter, Edwards built a lively group, its members working in diverse areas of reproductive science that reflected his own broad interests and knowledge. Moreover, Edwards encouraged a spirit of open communication and egalitarianism, which extended across all three groups, with sharing of resources, space, equipment, knowledge, and ideas, as well as social activities.

Through the 1960s and 1970s, Edwards’ work was funded by the Ford Foundation via grants first to Parkes and then to Austin (71) to continue work on basic reproductive mechanisms, with an eye to developing new methods of fertility control, and he continued to pursue the immunology of reproduction. However, he also worked on egg maturation, collecting pig, cow, sheep, the odd monkey, and some human eggs. He showed that eggs of all these species would indeed mature in vitro, but that the eggs of larger animals simply needed a longer time than those of smaller ones, with human eggs taking up to 36 hours rather than the 12 hours or less erroneously reported by Pincus. These cytogenetic studies were reported in two seminal papers in 1965 (72,73), both of which are primarily concerned with understanding the kinetics of the meiotic chromosomal events during egg maturation. In its discussion, the Lancet paper displays a breathtaking clarity of vision as Edwards sets out a program of research that predicted the events of the next 20 years and beyond (Table II.1). Significantly, if not surprisingly given his research interests, the early study and detection of genetic disease is afforded a heavy focus compared with the slight emphasis on infertility relief.

Table II.1 Key points in the program of research laid out in the discussion to Edwards’ 1965 Lancet paper

| 1. Studies on non-disjunction of meiotic chromosomes as a cause of aneuploidy in humans |
| 2. Studies on the effect of maternal age on non-disjunction in relation to the origins of trisomy 21 |
| 3. Use of human eggs in in vitro fertilization (IVF) to study fertilization |
| 4. Study of culture methods for human eggs fertilized in vitro |
| 5. Use of priming hormones to increase the number of eggs per woman available for study/use |
| 6. Study of early IVF embryos for evidence of (ab)normality—especially aneuploidies arising prior to or at fertilization |
| 7. Control of some of the genetic diseases in man |
| 8. Control of sex-linked disorders by sex detection at the blastocyst stage and transfer of only female embryos |
| 9. Intracervical transfer of IVF embryos into the uterus |
| 10. Use of IVF embryos to circumvent blocked tubes |
| 11. Avoidance of a multiple pregnancy (as observed after hormonal priming and in vivo insemination) by transfer of a single IVF embryo |


* Five aims relating specifically to genetic disease.

b One aim relating specifically to infertility relief.
alleviation. This genetic focus continues in his research papers over the next four years. Thus, within three years, working with his graduate student Richard Gardner, he provided proof of principle for preimplantation genetic diagnosis (PGD) in a paper on rabbit embryonic sexing published in 1968 (74), a paper that was to anticipate the development of PGD clinically by some 22 years (75). Likewise, working with the Cambridge geneticist Alan Henderson, Edwards was to develop his “production line theory” of egg production to explain the origins of maternal aneuploidy in older women. Thus, the earliest eggs to enter meiosis in the fetal ovary were shown to have more chiasmata and to be ovulated earlier in adult life than those entering meiosis later in fetal life (76,77).

THE PROBLEM OF FERTILIZATION OF THE HUMAN EGG

Notwithstanding his broad range of scientific interests, Edwards’ ambitions to achieve IVF in humans remained undiminished. In 1966, this was no trivial task, having been accomplished convincingly only in rabbits and hamsters (78,79). In trying to achieve this aim, he was engaging in two struggles: the first being simply but critically the continuing practical difficulty in obtaining a regular supply of human ovarian tissue. Local Cambridge sources proved unreliable and Molly Rose was now two to three hours’ drive away in London; so, during the summer of 1965, Edwards turned to the U.S.A. for help and approached Victor McKusick, a leading American cytogeneticist at the Johns Hopkins University. There he initiated his long-standing contact with Howard and Georgeanna Jones in obstetrics and gynecology (80). The supply of American eggs they generated during his six-week stay allowed him to confirm the maturation timings that were published the same year.

However, it was the second scientific struggle that was then occupying most of his attention, namely that in order to fertilize these _in vitro_-matured eggs, he had to “capacitate” the spermatozoa, a final maturation process that spermatozoa undergo physiologically in the uterus and that is essential for the acquisition of fertilizing competence. The requirement for sperm capacitation had been discovered in the early 1950s by Austin, and independently by M.C. Chang (81,82). Failing to achieve this convincingly at Johns Hopkins, he made a second transatlantic summer journey in 1966 to visit Luther Talbot and his colleagues at Chapel Hill. He tried a variety of ways (83) to overcome the problem of “sperm capacitation,” one of the most ingenious of which was to construct a 2.5 cm-long chamber from a nylon tube, plugged at each end, and with holes drilled in the walls that were encased in panels made of Millipore membrane (84). The chamber, which had a short thread attached to it, fitted snugly inside the inserter tube of an intrauterine device and so could be placed into the volunteer woman’s uterus intracervically at mid-cycle, where it sat for up to 11 hours before being recovered by gently pulling on the thread, exactly as was being done routinely for the insertion and removal of intrauterine devices. By placing spermatozoa within the chamber, the membrane of which permitted equilibration of its contents with uterine fluid, he hoped to expose them to a capacitating environment. However, this ingenious approach, like the many others, failed—in this case most probably because the chamber itself induced an inflammatory response or a local bleed. For all the ingenuity of his various experimental approaches to achieving capacitation, and despite the occasional evidence of early stages of fertilization using such spermatozoa, no reliable evidence for the completion of the process was forthcoming. Then, in 1968, both struggles began to resolve.

THE MEETING WITH PATRICK STEPTOE

Patrick Steptoe (1913–1988; Figure II.7) had been a consultant obstetrician at Oldham General Hospital since 1951 (85), where for several years he had been pioneering the development and use of the laparoscope in gynecological surgery (85,86). Much to his frustration, his progress had fallen on the largely deaf ears of the conservative gynecological hierarchy, and indeed incited considerable opposition and some outright hostility (87). Edwards’ claimed that he was scanning the medical and scientific journals in the library, and in a “eureka” moment occurring in “one autumn day in 1967” (2), came across a paper by Steptoe describing his experiences with laparoscopy (2,85,88). Edwards goes on to describe how he rang Steptoe to discuss a possible collaboration, but was “warned off” Steptoe by London gynecological colleagues (2,89). This warning and the daunting prospect of collaboration in far-away Oldham deterred him from following through. Finally, Edwards reported actually meeting Steptoe the following spring of 1968 at a meeting at the Royal Society of Medicine, at which, ironically, Edwards was talking about his work on immuno-reproduction, not his attempts at IVF.
The Steptoe paper that Edwards found that day in the library was cited in his later tributes to the then deceased Steptoe (85,88) as being a *Lancet* paper entitled “Laparoscopy and ovulation” (90). However, these later recollections do not withstand scrutiny. Thus, the *Lancet* paper cited was published in October 1968, but their first meeting was in fact earlier that year, on Wednesday February 28, 1968, at a joint meeting of the Section of Endocrinology of the Royal Society of Medicine with the Society for the Study of Fertility held at 1 Wimpole Street (1,91). Moreover, according to Steptoe (92), they had already commenced collaborating prior to October 1968; indeed, their first paper together was submitted for publication later that year in December 1968 (see next section). Clearly, the paper read by Edwards must have been another, earlier than October 1968, one that preceded February 1968 by several months. The “paper” by Steptoe that Edwards most likely saw was his book on gynecological laparoscopy (1,86,93,94), and the feature that probably caught his attention, according to two earlier accounts (1,2,89), was his realization that laparoscopy could provide a way of recovering capacitated spermatozoa from the oviduct by flushing with a small volume of medium: “a practical way … of letting spermatozoa be in contact with the secretions of the female tract” (2). Indeed, Edwards says he actually rang Steptoe to ask whether this really was possible and was reassured by him that this was the case. Steptoe explicitly lays out this possibility in his book (86). Thus, on page 27 he reports: “By means of laparoscopy, Sjovall (1964) has carried out extended post-coital tests and has recovered spermatozoa from the fimbriated end of the tubes …”; and on page 70 he writes: “An extended post-coital test can be done by aspirating fluid from the tubal ostium …” Steptoe’s book arrived in the Cambridge University library in March 1967 (1) and it is possible that Edwards’ attention was drawn to the book by a review of it in the *British Medical Journal* on November 11, 1967 (1,95). This conclusion conflicts with the later memories of Edwards (85,88) that he contacted Steptoe initially because of his ability to recover eggs laparoscopically. However, it is possible that by time they met, some six months later, this had become more of a concern to Edwards, given the emerging reports of the failure of *in vitro*-matured rabbit eggs to produce viable embryos. Indeed, a letter, written admittedly on July 30, 2003, by Eliot Philipp, recalls that at the actual meeting Edwards had said it was eggs that he wanted Steptoe to recover (Figure II.8), albeit for making human stem cells (96), an enduring interest of Edwards.

**FERTILIZATION OF THE HUMAN EGG ACHIEVED AT LAST**

Despite the initiation of the collaboration with Steptoe, the actual solution to the capacitation problem existed nearer to home than Oldham, in the laboratories shared with Austin. In the early 1950s, Austin had co-discovered the requirement for sperm capacitation (81,82), and after his appointment to the Cambridge chair, Austin’s first graduate student (1967–1972) was Barry Bavister, who set

---

**Figure II.8**  Extract from a letter, written on July 30, 2003, by Eliot Philipp to Edwards, recalling his memories of the words used at the first meeting between Edwards and Steptoe (courtesy of the Edwards family).
Robert G. Edwards and the thorny path to the birth of Louise Brown: A history of in vitro fertilization and embryo transfer

Table II.2 Summary of data

<table>
<thead>
<tr>
<th>Egg type</th>
<th>Experimental</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initially assigned</td>
<td>56</td>
<td>17</td>
</tr>
<tr>
<td>Survived</td>
<td>54/56</td>
<td>17/17</td>
</tr>
<tr>
<td>Matured to metaphase II</td>
<td>34/56</td>
<td>7/17</td>
</tr>
<tr>
<td>Evidence of sperm penetration</td>
<td>18/34</td>
<td></td>
</tr>
<tr>
<td>Sperm within the zona pellucida</td>
<td>6/18</td>
<td></td>
</tr>
<tr>
<td>Sperm inside the zona pellucida (~7 hours post-insemination)</td>
<td>5/18</td>
<td></td>
</tr>
<tr>
<td>Evidence of pronuclei (~11 hours post-insemination)</td>
<td>7/18</td>
<td>0/7</td>
</tr>
<tr>
<td>With two pronuclei</td>
<td>2/18</td>
<td></td>
</tr>
</tbody>
</table>


to work to try and define the factors influencing the capacitation of hamster spermatozoa in vitro. By 1968, Bavister had discovered a key role for pH, showing how higher rates of fertilization could be obtained by simply increasing the alkalinity of the medium (97). Edwards seized on this observation and co-opted Bavister to his project. That proved to do the trick, and in December 1968 Edwards, together with Bavister and Steptoe, submitted the paper to Nature in which IVF in humans was described convincingly for the first time (38).

This 1969 Nature paper makes modest claims. Only 18 of 56 eggs assigned to the experimental group showed evidence of “fertilization in progress,” of which only two were described as having the two pronuclei to be expected if fertilization were occurring normally (Table II.2). However, like Edwards’ other papers, this one is a model of clarity, describing well-controlled experiments, cautiously interpreted. Despite the relatively small numbers, this paper convinced eventually, although some doubts were expressed at the time (37, 98). That this paper convinced where previous claims had failed (99–104) was precisely because the skilled hands and creative intellect that were behind it are so evident from its text.

The provenance of the eggs described in the 1969 paper is not immediately clear from the paper itself. All were obtained by in vitro maturation after ovarian biopsy, but in addition to Steptoe’s co-authorship, four other gynecologists are thanked in the acknowledgements section of the paper: Molly Rose, Norman Morris (1920–2008; Professor of Obstetrics and Gynaecology at Charing Cross Hospital, London from 1958 to 1985 [105]), Janet Bottomley (1915–1995; Consultant Obstetrician and Gynecologist at Addenbrooke’s Hospital, Cambridge from 1958 to 1976), and Sanford Markham (b. 1934; Chief of the Section of Obstetrics and Gynaecology at the U.S. Air Force Hospital, South Ruislip, to the northwest of London from 1967 to 1972). An analysis described by Johnson (1) reasonably concludes that those eggs described in the paper as “undergoing fertilization” were provided in roughly equal numbers by Rose and Steptoe.

However, with Steptoe now on board, Rose no longer featured as a supplier of eggs (2). While the initial attraction of laparoscopy for Edwards had been the recovery of capacitated spermatozoa from the oviduct, once working with Steptoe, he rapidly exploited the wider possibilities for the recovery of in vivo-matured eggs from the ovary (90). Indeed, the 1969 paper includes the following statement: “Problems of embryonic development are likely to accompany the use of human oocytes matured and fertilized in vitro. When oocytes of the rabbit and other species were matured in vitro and fertilized in vivo, the pronuclear stages appeared normal but many of the resulting embryos had subnuclei in their blastomeres, and almost all of them died during the early cleavage stages … When maturation of rabbit oocytes was started in vivo by injecting gonadotropins into the mother, and completed in the oviduct or in vitro, full term rabbit fetuses were obtained” (98). The paper goes on to discuss how the use of hormonal priming to stimulate intrafollicular egg maturation might be achieved and reports: “Preliminary work using laparoscopy has shown that oocytes can be recovered from ovaries by puncturing ripening follicles in vivo …”

Through these preliminary collaborative studies, Edwards and Steptoe were already building a research partnership. Although both had very different personalities and brought very different skills to the project, they shared energy, commitment, and vision. Both were also marginalized by their professional peers, a marginalization that also perhaps helped to cement their partnership (3). With the paper’s publication, announced to the media on St. Valentine’s Day (106), all hell was let loose. The impossible tangle of TV cables and pushy reporters trying to force their way up the stairs to the fourth floor laboratories proved a major disruption to the physiological laboratory in general and to the members of the Marshall laboratory in particular. It was something that was to recur episodically over the next 10 years.

THE BATTLES BEGIN

However, 1969 seemed to be a good year for Edwards. Not only did IVF succeed at long last and his partnership with Steptoe seemed set to flourish, but also so impressed were the Ford Foundation with his work that in late 1968 they had established, at Austin’s prompting (107), an
endowment fund with the University of Cambridge to cover the salary cost of a Ford Foundation Readership (a halfway step to a professorship [108]). Elated by Edwards' promotion and their achievement, Edwards and Steptoe pressed on, with the latter's laparoscopic skills coming to the fore, first in 1970 with the collection of in vivo-matured eggs from follicles after mild hormonal stimulation (4,109), and then achieving regular fertilization of these eggs and their early development through cleavage to the blastocyst stage (110–112). So well was the work going that in late 1970 and early 1971 they confidently applied to the U.K. Medical Research Council (MRC) for long-term funding (2).

However, any illusions that Edwards may have had that their achievements would prove a turning point in his fortunes were soon shattered. The hostility to his work of much of the media coverage in 1969 heralded the dominant pattern of scientific and medical responses for the next 10 years and resulted just two months later in the MRC rejecting the grant application (3). The practical consequences of this rejection were profound—both psychologically and physically—not least that for the next seven years, Edwards and Purdy shuttled on the 12-hour round trip between Cambridge and Oldham, Greater Manchester, paradoxically just north of his schoolboy haunts of Gorton, where the two of them had set up a small laboratory and clinic in Dr. Kershaw's cottage hospital (113), all the while leaving Ruth and his five daughters in Cambridge. The one bright feature in undertaking this heroic task was the unswerving financial support provided by an American heiress—Lillian Lincoln Howell (71)—at least ameliorated the MRC decision.

The professional attacks on Edwards and his work took a number of forms (3), and one must try to make a mental time trip back to the 1960s and 1970s to understand their basis. Despite the nature of the political and religious battles to come in the 1980s, his scientific and medical colleagues did not then focus on the special status of the human embryo as an ethical issue. Ethical issues were raised professionally, but took quite a different form. It is perhaps difficult now to comprehend the complete absence of infertility from the consciousness of most gynaecologists in the U.K. at the time, of whom Steptoe was a remarkable exception (85). Indeed, even Edwards' strong commitment to treating infertility came to the fore only after he had teamed up with Steptoe, with his previous priority being the study and prevention of genetic and chromosomal disorders.

In the several reports from the Royal College of Obstetricians and Gynaecologists (RCOG) and the MRC during the 1960s examining the areas of gynaecological ignorance that needed academic attention, infertility simply did not feature (52,53). Overpopulation and family planning were seen as dominant concerns and the infertile were ignored as, at best, a tiny and irrelevant minority and, at worst, as a positive contribution to population control. This was a values system that Edwards did not accept (114), and the many encouraging letters he received from infertile couples spurred him on and provided a major stimulus to his continued work later, despite so much professional and press antagonism. For his professional colleagues, however, the fact that infertility was not seen as a significant clinical issue meant that any research designed to alleviate it was viewed not as experimental treatment, but as using humans in experiments. Given the sensitivity to the relatively recent Nazi "medical experiments," the formal acceptance of the Helsinki Declaration (115,116), and the public reaction and disquiet surrounding the recent publication of "human guinea-pigs" (117), this distinction was critical. The MRC, in rejecting the grant application, took the position that what was being proposed was human experimentation, and so were very cautious, emphasizing risks rather than benefits, of which they saw few if any (3,5).

Edwards and Steptoe were also attacked for their willingness to talk with the media. It is difficult nowadays, when the public communication of science is embedded institutionally, to understand how damaging to them this was. The massive press interest of the late 1960s was unabated in the ensuing years, and so Edwards was faced with a choice: either he could keep his head down and allow press fantasies and speculations to go unanswered and unchallenged, or he could engage, educate, and debate. For him this was no choice, regardless of the consequences professionally (32). His egalitarian spirit demanded that he trust common people's common sense. His radical political views demanded that he fought the corner of the infertile: the underdog with no voice. The Yorkshireman in him relished engagement in the debate and argument. In Edwards and Sharpe (114), he sets out his reasons for public engagement and acknowledges the risk to his own interests:

Scientists may have to make disclosures of their work and its consequences that run against their immediate interests; they may have to stir up public opinion, even lobby for laws before legislatures (114).

And risky it was. One of the scientific referees on their MRC grant application started his referee's report by declaring the media exposure distasteful:

Dr. Edwards feels the need to publicise his work on radio and television, and in the press, so that he can change public attitudes. I do not feel that an ill-informed general public is capable of evaluating the work and seeing it in its proper perspective. This publicity has antagonised a large number of Dr. Edwards' scientific colleagues, of whom I am one (3).

Edwards' pioneering role in the public communication of science proved to be disadvantageous to his work.

The Edwards and Sharpe (114) paper is a tour de force in its survey of the scientific benefits and risks of the science of IVF, in the legal and ethical issues raised by IVF, and in the pros and cons of the various regulatory responses to them. It sets out the issues succinctly and anticipates social
responses that were some 13–19 years into the future. Edwards built on his strong commitment to social justice based on a social ethic in subsequent years, as he engaged at every opportunity with ethicists, lawyers, and theologians, arguing, playing “devil’s advocate” (literally, in the eyes of some), and engaging in what would now be called practical ethics as he hammered out his position and felt able to fully justify his instincts intellectually (118). However, the establishment was, with few exceptions, unwilling to engage seriously in ethical debates (118,119) in advance of the final validation of IVF that was to come in 1978 with the birth of Louise Brown (Figure II.9) (120).

### THE BIRTH OF LOUISE BROWN

It is difficult now to comprehend the sheer magnitude of the task facing Edwards, Steptoe, and Purdy in 1969. Not only did they suffer almost complete isolation from their peers, they also faced a massive scientific and clinical mountain to climb to get from a fertilized egg to a baby, given the paucity of knowledge at the time (see Table II.3). In fact, their progress on reaching the point where transfer of embryos was possible (stage 9 in Table II.3) was impressively rapid, with the first embryo transfer being attempted in December 1971, just two years from the start of their collaboration. This rapid progress was achieved probably because the end point of each task from stage 1 to stage 9 was easily measurable in relation to controlled changes made to the protocols (112). However, for the 97 women who underwent laparoscopy between 1969 and December 1971, when egg recovery, fertilization, and in vitro culture were being perfected, there was no chance of a pregnancy, and so they were “experimental subjects,” as the MRC had claimed. Moreover, 76 of them (27% of the total number of women who volunteered as patients between 1969 and 1978) did not subsequently undergo embryo transfer attempts in Oldham up to 1978 (5). However, all the evidence suggests that these patients were well informed about the risks and benefits (5), but nonetheless they, as much as Edwards, Purdy, and Steptoe, deserve recognition for their pioneering role in the development of IVF.

After the first transfer in December 1971, most viable embryos were transferred, with a total of 112 transfers occurring between that first one and the last in June 1978 (4,112). Once transfer was being attempted, the task became much more difficult, however, and this difficulty was behind the delay in achieving a successful pregnancy. There were essentially two types of problem to be grappled with. The first was that multiple features of the system could have been responsible for the failure to establish a pregnancy: the transfer technique and timing (both of which had proved difficult to get right in cattle) (120), the quality of the embryos, and the receptivity of the endometrium. Moreover, varying the latter two of these systematically was difficult, and, given the absence of a reliable and sensitive test for human chorionic gonadotropin until late in 1977, there was no immediate way of assessing the impact of any changes that were made. The second problem was the suspicion that the endocrine conditions established as being ideal for the production of eggs and embryos may have been deleterious for the receptivity of the endometrium. Indeed, it was this latter suspicion that drove most of their experimental variations to the treatment schedules, and that ultimately resulted in the two successful

---

**Figure II.9** Louise Brown holding the thousandth Bourn Hall baby, 1987 (courtesy of Bourn Hall Clinic).

---

**Table II.3** Some of challenges that had to be overcome before the first successful live births after in vitro fertilization and embryo transfer were achieved

| 1. Technical aspects of follicle aspiration laparoscopically (“new suction gadget”) |
| 2. Ovulation induction |
| 3. Timing of laparoscopy in relation to induction of ovulation |
| 4. Ovarian stimulation |
| 5. Cycle monitoring |
| 6. Oocyte culture |
| 7. Sperm preparation |
| 8. Insemination procedure: medium and timing |
| 9. Culture for embryo cleavage: medium and assessment |
| 10. Technical aspects of embryo transfer, including route of transfer, medium, and timing |
| 11. Luteal support |
| 12. Monitoring of early pregnancy |

Oldham pregnancies, both of which came, heroically, from single egg collections in natural cycles (4,112).

Only with the live births did the U.K. social, scientific, and medical hierarchies, such as the MRC, the RCOG, the British Medical Association, the Royal Society, and Government moved, albeit gradually, from their almost visceral reactions against IVF and its possibilities to serious engagement with the issues (121). Thus, both the MRC and the RCOG started to move to consider funding of work on IVF, perhaps somewhat surprisingly, given that only two live births resulted from a total of 112 transfers (4), although again the MRC declined to fund a second grant proposal from Edwards. Moreover, the National Health Service declined to provide facilities in Cambridge for Edwards and Steptoe to relocate to on Steptoe’s retirement from Oldham, hence the setting up of the Bourn Hall clinic in 1982. The Thatcher Government of the time started to seriously consider the issues and set up the Warnock committee of enquiry in 1982 that reported in 1984, to a storm of parliamentary criticism, which Edwards and others had to battle to turn around over the next five years with a fierce campaign of both public and parliamentary education to counter the increasingly shrill voice of the anti-embryo research lobby (121,122). In addition, Edwards’ personal battles continued, with legal suites issued during the 1980s against both the British Medical Association and various members of the national press for defamation. Thus, it was not until 1989, 24 years after Edwards’ 1965 visionary paper in *The Lancet*, 20 years after IVF had first been described, and 11 years after the birth of Louise Brown that the U.K. Parliament finally gave its stamp of approval to his vision.

However, Edwards’ role was realized and recognized professionally at last by the awards of fellowships of the RCOG in 1984 and of the Royal Society in 1983 (and an FRS for Steptoe followed in 1987). But despite being awarded the Albert Lasker prize in 2001, the Nobel Prize and a knighthood did not come his way until 2010—some 40 years after the *Nature* paper that started the whole IVF story in earnest.

**DISCUSSION**

This chapter describes some of the early years of Edwards’ life and work in order to provide a context for the events leading up to the 1969 *Nature* paper describing IVF and the final validation of the claims made in that paper with the birth of Louise Brown in 1978. It is evident even from the earliest stages of his late entry into research that Edwards is a man of extraordinary energy and drive, qualities sustained throughout his long career, as witnessed in his prodigious output of papers between 1954 and 2008 (32). Indeed, several of the referees on the unsuccessful 1971 MRC grant application specifically criticized his “overenthusiasm,” doubting that he could achieve the program he sets out therein as “too ambitious” (3). Tenacity of purpose comes through clearly in Edwards’ work, a trait he was inclined to attribute to his Yorkshire origins, but that may also have been fueled by his working-class determination to show himself to be as good as the next (wo)man. The influences of Waddington’s Edinburgh Institute, of Waddington himself, and of his supervisor, Alan Beatty, on Edwards’ interests and values are also clear from the dominant role that developmental genetics played in his thinking, especially until the time he met Steptoe. Indeed, from examination of Edwards’ papers and interests, his passionate conversion to the cause of the infertile seems directly attributable to Steptoe’s influence. Admittedly, Edwards’ forays into immuno-reproduction did involve consideration of immunological causes of infertility, but these were more usually of interest to him as models for developing new contraceptive agents. Indeed, Edwards was as captured as most reproductive biologists of the time by the 1960s’ consensus on the need for better methods of world population control. This position was understandable given the reality of those concerns, as is demonstrated now in the problem of global warming that is attributable at least in part to a failure to control population growth. It is a measure of his imagination and empathy that he could grasp so rapidly Steptoe’s understanding of the plight of the infertile and so flexibly incorporate this understanding into his plans. That empathy clearly reflects his underprivileged origins, with his espousal of the cause of the junior, the disadvantaged, the ill-informed, and the underdog being a thread running through his career. Edwards can be very critical, but I have found no one who can remember him ever being nasty or vindictive. Even when he disagrees with someone passionately, he never loses his respect for them as people. That Steptoe tapped into this sentiment is clear.

The way in which Edwards met Steptoe has been absorbed into folklore, but an examination of the evidence seems to warrant some revision to commonly held later reminiscences. It remains uncertain exactly which publication(s) by Steptoe it was that Edwards read in 1967, but seems likely that he did read Steptoe’s book. Thus, it was spermatozoa, not eggs, that were exercising Edwards in 1967, and it was the problem of sperm capacitation, not egg retrieval, to which Steptoe and his laparoscope seemed to offer a solution in 1967. The book is the only place that this issue is specifically addressed. Their actual meeting at the Royal Society of Medicine in 1968 is also re-evaluated: Edwards was an invited speaker lecturing about his work on immuno-reproduction; so paradoxically, what has been seen as a sidetrack to his main work was, albeit serendipitously, the reason for their actual meeting.

The early collaboration between them involved the recovery of ovarian biopsies, just like those Rose and others had been providing. However, the attractions of pre-ovulatory follicular egg recovery were already clear to them both by 1968, and became, with embryo replacement, the central planks of their partnership. Steptoe and Edwards were in many ways an unlikely partnership. Their personal styles were very different, and there are clear hints in his writings that Edwards found their early days together difficult. But like most successful partnerships, their differences were sunk in a mutual respect for the other’s pioneering skills and willingness to take on
the established conventions. In Jean Purdy, they also had a partner who worked quietly away in the background, smoothing the bumps on the path of their work together (Figure II.10) (113).

However, it remains Edwards’ extraordinary foresight that marks him out so distinctively. His combination of vision and intellectual rigor is evident not just in his work on stem cells, PGD, and, with Steptoe, infertility, but also in his pioneering work in the public communication of science, in how ethical discourse about reproduction is conducted, in consideration of regulatory issues, and in the dissemination of IVF internationally, the latter largely a consequence of his key role in both the establishment of the European Society for Human Reproduction and Embryology in 1984 and in the founding of five journals: *Human Reproduction* (in 1986), *Human Reproduction Update* and *Molecular Human Reproduction* (both in 1996), *Reproductive BioMedicine Online* (in 2000) and *Reproductive BioMedicine and Society* (in 2015). The epithet “the father of assisted reproduction” is surely deservedly appropriate.

**ACKNOWLEDGMENTS**

I thank the Edwards family for their help in writing this account, plus Kay Elder, Sarah Franklin, Nick Hopwood, and Allen Packwood for their unerring wisdom and help, and Barry Bavister, Richard Gardner, Roger Gosden, David Griffin, Ginny Papaioannou, Barbara Rankin, Carol Readhead, Sarah Howlett, Pat Tate, and Frank Webb for contributing their own memories from their own papers and for correcting mine. However, I take full responsibility for the contents of this article. I thank the Edwards family for permission to reproduce Figures II.1, II.2, II.4, II.5, and II.8, Julio Sirlin for permission to reproduce Figure II.3, Barbara Rankin for permission to reproduce Figure II.6, Andrew Steptoe for permission to reproduce Figure II.7, and Bourn Hall Clinic for permission to reproduce Figures II.9 and II.10. The research was supported by grants from the Wellcome Trust (088708 to Nick Hopwood, Martin Johnson et al., 100606 to Sarah Franklin, and 094985 to Allen Packwood and Martin Johnson), which otherwise had no involvement in the research or its publication.

**REFERENCES**


**Figure II.10** Edwards, Purdy, and Steptoe at Bourn Hall, 1981 (courtesy of Bourn Hall Clinic).
Robert G. Edwards and the thorny path to the birth of Louise Brown: A history of in vitro fertilization and embryo transfer


New guidelines for setting up an assisted reproduction technology laboratory

JACQUES COHEN, MINA ALIKANI, ANTONIA GILLIGAN, and TIM SCHIMMEL

There are a number of ways to set up and operate a successful assisted reproduction technology (ART) laboratory; one set-up may have little in common with another but prove to be equally successful. This is important to remember as one ventures into establishing a new clinic. Facilities for ART range from a makeshift in vitro fertilization (IVF) laboratory with a minimum of equipment to a fully equipped laboratory specifically designed for ART and with additional space dedicated to clinical care and research. This chapter does not cover makeshift laboratories, which may incorporate retrieval and transport of gametes and embryos from other locations. While such models can be successful under some circumstances, compelling evidence showing that they produce optimal results is still lacking (1,2). Both IVF and intracytoplasmic sperm injection (ICSI) can be applied to transported oocytes, and in certain situations “transport IVF” is a welcome alternative for those patients whose reproductive options have been limited by restrictive governmental regulations (3,4). This chapter discusses the more typical purpose-built, all-inclusive laboratories that are adjacent or in close proximity to oocyte retrieval and embryo transfer facilities, with an emphasis on the special problems of construction. For choices of culture system, culture medium, supplementation, viability assays, and handling and processing of gametes and embryos, including freezing and vitrification, the reader is referred to other relevant chapters in this textbook.

PERSONNEL AND EXPERIENCE

While the environment, physical plant, and equipment require special consideration in the design of an integrated gamete and embryo culture facility, it is the staff that will carry out the procedures and is essential to the success of the entire operation. Successful clinical practice in general, and ART in particular, is almost entirely dependent on the skill and experience level of medical and laboratory personnel. For the laboratory staff, enthusiasm is another key factor to success, especially because there are still few formal teaching and skills examination programs in place for a specialty in ART. Most clinical embryologists are trained using an apprenticeship program, but such institutions are rare and there are no internationally accepted guidelines. Good clinical outcome requires a cautious and rational assessment of individual abilities, so laboratory staff, directors, and embryologists must consider their experience in the context of what will be required of them (5).

This chapter aims to provide information necessary for experienced practitioners to set up a new laboratory. Setting up a new laboratory or thoroughly renovating an existing facility is very much an art, as is the practice of ART itself. We do not recommend that new laboratories and ART clinics are built by administrators, engineers, or architects without considerable input from experienced embryologists.

Programs should develop a strong (though friendly) system of tracking individual performances for crucial clinical and laboratory procedures such as embryo transfer efficiency, ICSI, and biopsy proficiency, among others. Certain regulatory bodies such as the College of American Pathologists (CAP) in the U.S.A. and the Human Fertilisation and Embryology Authority (HFEA) in the U.K. provide guidelines and licensing for embryologists, sometimes even for subspecialties such as the performance of ICSI, the practice of embryo biopsy, and directing IVF and andrology laboratories. So far, such licensing has done little more than provoke debate, because licensing does not necessarily guarantee skill (or success) and the licenses are not valid across borders from one country to another.

 Tradition also plays its role. For example, in some Asian countries embryoology directors are usually medical professionals. Thus academic titles are often seen as being more important than actual qualifications. What then qualifies someone to be a laboratory director or an embryologist? The answer is not a simple one. In general, current licensing authorities including the American Board of Bioanalysis (ABB) consider individuals trained in general pathology or reproductive medicine and holding an MD degree as well as individuals holding a PhD degree qualified to be laboratory directors if they meet some other requirements. However, pathologists do not necessarily have experience in gamete and embryo cell culture, and some reproductive medicine specialists, such as urologists and immunologists, may have never worked with gametes and embryos. It is possible for a medical practitioner to direct a laboratory in certain countries without ever having practiced gamete and embryo handling! “Eppur si muove” (“And yet it moves”), as Galileo said when condemned by the Roman inquisition for the heresy of accepting Copernican astronomy. Once there are rules, even silly ones, it may be hard to change them.

EMPIRICAL AND STATISTICAL REQUIREMENTS FOR STAFF

There is considerable disagreement about what should be required experience for embryologists. Hands-on experience in all facets of clinical embryology is an absolute requirement when starting a new program. Even highly
experienced experimental embryologists and animal scientists should be directly supervised by experienced clinical personnel. The period during which close supervision must continue depends on the types of skills required, the daily caseload, and time spent performing procedures. Clearly, performing 100 cases over a one-year period is a very different circumstance than performing the same number over six weeks; the period of supervision then should be adjusted accordingly.

The optimal ratio of laboratory staff to the expected number of procedures is debatable, and unfortunately, economics play an all too important role here. However, with the incorporation of new technologies and treatment modalities in routine care, the complexity of IVF laboratory operations has increased substantially over the past decade, in turn requiring more careful consideration of staffing levels (6). According to some calculations, while a “traditional” IVF cycle required roughly nine personnel hours, a contemporary cycle can require up to 20 hours for completion. Thus the number of embryologists required for safe and efficient operation of the laboratory has also increased. Recently, based on a comprehensive analysis of laboratory tasks and their complexity, an Interactive Personnel Calculator was introduced to help laboratory directors and administrators determine staffing needs (6). Overall, it is safe to say that the ratio of laboratory staff to caseload should be high so that embryologists can not only safely perform procedures but dedicate time to quality control and continued education and training in order to maintain the high standards required for success. The challenge of keeping these standards within national health systems or in the face of insurance mandates that must provide a wide range of services on a minimal budget is real, but should not be insurmountable. Needless to say, patients usually do not benefit from such constraints, as a comparison of results in different health service systems in Western countries would suggest. There are limitations to such comparisons, but live births per embryo and cumulative data from fresh and cryopreservation cycles are considered objective assessments (7).

The job description for the embryologist ideally includes all embryology and andrology tasks, except for medical and surgical procedures. Embryologists are often involved in other important tasks as well, including patient management, follicular monitoring, genetic counseling, marketing, and administration. However, it should be realized that these tasks seriously detract from their main responsibilities. First and foremost, the embryologist’s duty is to perform gamete and embryo handling and culture procedures. Secondly, but equally important, the embryologist should maintain quality control standards, both by performing routine checks and tests and by maintaining detailed logs of incidents, changes, unexpected events, and corrective measures. Across all these duties, the following seven positions can be clearly defined: director, supervisor, senior embryologist, embryologist, trainee, assistant, and technician. There may also be positions for others to do preimplantation genetic diagnosis, research, quality control supervision, or administrative work. Obviously, not all of these separate positions are necessary for smaller centers and important tasks can be combined.

Although a seemingly unimportant detail, one of the most important jobs in the IVF laboratory at Bourn Hall Clinic in Cambridge, U.K., during the first few years of operation was that of a professional witness and embryology assistant. This position was the brainchild of Jean Purdy, the third partner who was involved in the work that led to the birth of Louise Brown. The embryology assistant effectively enforced and oversaw the integrity of the chain of custody of gametes and embryos during handling, particularly when large numbers of patients were being treated simultaneously. The “witness” also ensured that embryologists performed only those procedures for which they were qualified. Interestingly, recent literature suggests that this crucial concept has not been universally and fully understood or adopted by all IVF laboratories. In one group of laboratories (8), “limited and consequently virtually ineffective” witnessing processes were only abandoned in favor of a more robust witnessing program after implementation of a failure mode and effects analysis (FMEA) showed a high risk of error in gamete and embryo identification. The authors stated that, “Only after FMEA optimization has the witness embryologist been formally recognized as a committed role, specifically trained for witnessing shift work.” Hopefully, the publication of this and other similar studies (9) that show the effectiveness of a witnessing system will encourage more laboratories to re-examine their practices and allocate adequate resources to ensuring the safety and efficiency of all procedures performed by the laboratory.

**FACILITY, DESIGN, AND BUDGET**

In the early days of IVF some clinics were built in remote areas, based on the premise that environmental factors such as stress could affect the patient and thereby the outcome of treatment. Today’s laboratories are commonly placed in city centers and large metropolitan areas in order to service large populations locally. It is important that patients understand that there have been millions of others like them before and that in general IVF is a routine, though complex, medical procedure. It is clear that the choice of a laboratory site is of great importance for a new program. The recent development of better assays for determining the baseline quality of the environment facilitates site selection. There is now awareness that some buildings or building sites could be intrinsically harmful to cell tissue culture (10–12). The direct effect of poor air quality and the presence of volatile organic compounds (VOCS) on IVF outcomes has been demonstrated by recent studies of novel filtration systems and other countermeasures (13,14). A laboratory design should be based on the anticipated caseload and any subspecialty. Local building and practice permits must be assessed prior to engaging and completing a design. There are five basic types of design:

1. Laboratories using only transport IVF
2. Laboratories adjacent to clinical outpatient facilities that are only used part of the time
3. Full-time clinics with intra-facility egg transport using portable warming chambers
4. Fully integrated laboratories with clinical areas
5. Moveable temporary laboratories

Before developing the basic design for a new laboratory, environmental factors must be considered. While air quality in modern laboratories can be controlled to a degree, it can never be fully protected from the exterior environment and adjoining building spaces. Designers should first determine if the building or the surrounding site is scheduled to undergo renovations, demolition, or major changes of any kind in the foreseeable future. City planning should also be reviewed. Historical environmental data and trends, future construction, and the ability of maintenance staff to maintain and service the IVF laboratory need to be determined. Activity related to any type of construction can have a significant negative impact on any proposed laboratory. Prevalent wind direction, industrial hazards, and general pollution reports such as ozone measurements should also be determined. Even when these factors are all deemed acceptable, basic air sampling and determination of VOC concentrations is necessary inside and outside the proposed building area. The outcome of these tests will determine which design requirements are needed to remove VOCs from the laboratory area. In most cases, an over-pressured laboratory (at least 0.10–0.20 inches of water) that uses a high number (7–15) of fresh air changes per hour is the best solution, because it also provides for proper medical hygiene. The laboratory walls and ceiling should have the absolute minimum number of penetrations. This generally requires a solid ceiling, sealed lighting, and airtight utility connections. Contrary to many vendors’ representations, commercial suspended ceilings using double-sided tape and clips are not ideal. Doors will require seals and sweeps, and should be lockable. Ducts and equipment must be laid out in such a way that routine and emergency maintenance and repair work can be performed outside the laboratory with minimal disruption to the laboratory. Air handling must not use an open plenum design. In the ideal case, 100% outside air with chemical and physical filtration will be used with sealed supply and return ducts.

While providing cleaner air, 100% outside air sourcing will maximize the life of a chemical filter and will provide a lower concentration of VOCs in the IVF laboratory’s air. In climates where temperatures routinely exceed 32°C with 85%-plus relative humidity, 100% outside air could result in an unacceptable level of humidity (>60%), which could allow mold growth. In these cases the use of limited return air from the lab is acceptable. A 50% outside air system with 15–30 total air changes per hour does work well and the relative humidity becomes very controllable. To place this in perspective, traditional medical operating room design calls for 10%–15% outside air.

The air supply equipment may supplement outside air with recirculated air, with processing to control the known levels of VOCs. On rare occasions, laboratories will require full-time air recirculation, while most may actually find the outside air to be perfectly clean at least most of the time. Outside air is often erroneously judged to be polluted without proper chemical analysis, while inside air is usually considered “cleaner” because it may “smell” better. In most laboratory locations, conditions are actually the reverse, and designers should not “follow their instincts” in these matters. Humidity must also be completely controlled according to climate and seasonal variation. The system must be capable of supplying the space with air at a temperature as high as 30°C–35°C at less than 40% relative humidity. Air inlets and outlets should be carefully spaced to avoid drafts that can change local “spot” temperatures, or expose certain equipment to relatively poor air or changes in air quality. Laminar flow hoods and micromanipulation workstations should not be located too close to air supply fixtures to avoid disruption of the sterile field and to minimize cooling on the microscope stage. Semi-enclosed workstations based on Class 2 cabinets or neonatal isolette incubators can be considered to optimize the work environment and bridge the gap between the incubator and the workstation. A detailed layout and assessment of all laboratory furniture and equipment is therefore essential prior to construction and has many other benefits.

Selection of an experienced and sub-specialized (and flexible) architect and a mechanical engineer for the project is essential. Confirm what their past experience has been in building biologically clean rooms. The use of “environmentally friendly” or “green” products has been suggested by some designers. The reliance on “natural” products does not ensure a clean laboratory. In one case, wood casework with a green label was found to be a major source of formaldehyde. Floor coverings using recycled vinyl and rubber were selected for their low environmental impact, without considering the significant release of trapped gases by the material.

Supervision of the construction is also critical. Skilled tradesmen using past training and experience may not follow all of the architect’s instructions. The general contractor and the builders must be briefed on why these novel construction techniques are being used. They must understand that the use of untested methods and products can compromise the project (and the payment of their fees!). Contractual agreement is recommended.

Just as the organization and flow of traffic in a world-class restaurant results in a special ambience where more than just the food is the attraction, appropriate modular placement of equipment ensures safety and comfort in the over-pressured IVF laboratory. Placement of stacks of incubators, gamete handling areas (laminar flow units or isolettes), and micromanipulation stations should minimize distances that dishes and tubes need be moved. Ideally, an embryologist should be able to finish one complete procedure without moving more than three meters in any direction; not only is this efficient, but also it minimizes accidents in a busy laboratory. Design and implementation of a work area incorporating product, gas and...
liquid nitrogen supplies, and a workstation, refrigerator, and incubators is feasible even without the embryologists having to walk between storage cabinets and equipment. Such a modular design can be duplicated multiple times within a larger air handling area allowing the handling of large numbers of gametes and embryos. For logistical reasons, sperm preparation and cryopreservation may be placed in adjacent areas. The number of modules can easily be determined by the expected number of cases and procedure types, the average number of eggs collected, and the number of embryologists expected to work simultaneously. Each person should be provided with sufficient workspace to perform all procedures without delay. Additional areas can contain simple gamete handling stations or areas for concentrating incubators. Cryopreservation and storage facilities are often located in a separate space, although this is not strictly necessary; if separated, these areas should always be adjacent to the main laboratory. Storage spaces could be separated further using closets or rooms with negative pressure. Another separate laboratory or module may contain an area for culture medium preparation, sterilization, and water treatment; however, the need for such an area is diminishing now that commercial manufacturers provide all the basic needs of an IVF laboratory. Administration should be performed in separate offices on a different air handling system from the main laboratories.

Last but not least, it is preferable to prepare semen in a separate laboratory altogether, adjacent to one or more collection rooms. The semen laboratory should have ample space for microscopes, freezing, and sterile zoning. Proper separation of patient samples during processing is essential and some elemental design features may be considered before the first procedures are carried out. Some thought should go into planning the semen collection area. This small room should be at the end of a hallway preferably with its own exit; it should be soundproofed, not too large, and with a sink. Clear instructions on how to collect semen for ART should be provided in the room. The room should also be adjacent to the semen preparation laboratory, preferably with a double-door pass-through for samples. This pass-through should have a signaling device so the patient can inform the embryologist that the sample is ready; it also permits male patients to leave the area without having to carry a specimen container.

**EQUIPMENT AND STORAGE**

A detailed list of equipment should be prepared and checked against the planned location of each item; it can later be used as the basis of maintenance logs. It is important to consider the inclusion of crucial equipment and spare tools in the laboratory design, to allow for unexpected malfunction. Similarly, two or more spare incubators should not be seen as excessive; at least one spare follicle aspiration pump and micromanipulation station (equipped with a laser) should also be included. There are many other instruments and equipment pieces the malfunction of which would jeopardize patient care, although some spares need not be kept on hand as manufacturers may have them available; however, such details need to be repeatedly checked as suppliers’ stocks continue to change. It may also be useful to team up with other programs or an embryology research laboratory locally so that a crucial piece of equipment can be exchanged in case of unexpected failure.

Some serious thought is needed when contemplating the number and type of incubators (for a comprehensive review, see [15]). The ratio of incubators to patient procedures depends on incubator size and capacity and it varies considerably from program to program. It is clear that the number and type of incubator, as well as the length and number of incubator door openings, affect results. In principle, the number of cases per incubator should be kept to a minimum; we prefer a limit of four cases per large standard box incubator. The smaller box incubators should not handle more than two to three cases. In benchtop incubators, the use of one dish slot per patient is not recommended. Several other incubators can be used for general purposes during micromanipulation and for other generic uses to limit further the number of incubator openings. Strict guidelines must be implemented and adhered to when maintaining distinct spaces for separating culture dishes or tubes of different patients. Tracking of incubators and even shelves within each incubator is recommended so their performance can be evaluated on an ongoing basis. Separate compartments within an incubator may be helpful and can be supplied by certain manufacturers. Servicing and cleaning of equipment such as incubators may have to be done when the laboratory is not performing procedures. Placement of incubators and other pieces of equipment on large castors may be helpful in programs where downtime is rare. Pieces of equipment can then be serviced outside the laboratory. New incubators and equipment pieces that come in contact with gametes and embryos must be “burned-in” or “off-gassed.” Protocols vary per equipment type and manufacturer.

When there are several options available to the laboratory designer, supply and evacuation routes should be planned in advance. One of the most susceptible aspects of ART is cryopreservation. In case of an emergency such as a fire or power failure, it may be necessary to relocate the liquid nitrogen-filled dewars without using an elevator, or to relocate the frozen samples using a temporary container. This may seem an extreme consideration, especially in the larger laboratories that stockpile thousands of samples, but plans should be made. It may be possible to keep a separate storage closet or space near the building exit, where long-term samples, which usually provide the bulk of the storage, can be kept; this would require repeated checking of a facility that is not part of the laboratory. Liquid nitrogen tank alarms with remote notification capability should be installed on all dewars holding gametes and embryos. The route of delivery of liquid nitrogen and other gas cylinders must be relatively easy, without stairways between the laboratory and the delivery truck, and should be sensibly planned in advance. Note that the flooring of this route is usually destroyed within months.

---
because of liquid nitrogen spills and wear caused by delivery containers, so the possibility of an alternative delivery corridor should be considered for these units.

Liquid nitrogen containers and medical gas cylinders are preferentially placed immediately adjacent to the laboratory in a closet or small, ventilated room with outside access. Pipes and tubes enter the laboratory from this room, and cylinders can be delivered to this room without compromising the laboratory area in any way. Providing liquid nitrogen and even liquid oxygen vapor to triple gas incubators is nowadays a preferred option since vapor is cleaner than compressed gas. This allows liquid nitrogen vapor to be pumped into the cryopreservation laboratory using a manifold system and minimal piping. Lines should be properly installed and insulated to ensure that they do not leak or allow condensation and conserve energy at the same time. Medical gases can be directed into the laboratory using pre-washed vinyl/Teflon-lined tubing such as fluorinated ethylene propylene, which has high humidity, temperature, and UV radiation stability. Lines should be properly marked every meter indicating the incubators supplied in order to facilitate later maintenance. Alternatively, solid manifolds made from stainless steel with suitable compression fittings can be used. Avoid the soldered or brazed copper lines used in domestic plumbing applications wherever possible; copper lining can be used but should be cleaned and purged for a prolonged period prior to use in the laboratory. Copper line connections should not be soldered as this could cause continuous contamination. This recommendation may conflict with existing building codes, but non-contaminating alternatives can be found. A number of spare lines hidden behind walls and ceilings should be installed as well, in case of later renovation or facility expansion.

Large programs should consider the use of exterior bulk tanks for carbon dioxide and liquid nitrogen. This removes the issues of tanks for incubators or cryopreservation. These tanks are located where delivery trucks can hook onto and deliver directly to the tank. Pressurized gas lines or cryogenic lines then run the carbon dioxide or liquid nitrogen to the IVF laboratory for use.

Placement of bulky and difficult pieces of equipment should be considered when designing doorways and electrical panels. Architects should be fully informed of all equipment specifications to avoid the truly classic doorway mistake. Emergency generators should always be installed, even where power supplies are usually reliable. The requirements can be determined by an electrical engineer. Thankfully, these units can be removed from the laboratory, but must be placed in well-ventilated areas that are not prone to flooding. Additional battery “uninterruptible power systems” may be considered as well, but are of very limited capability. Buildings should also be checked for placement of the main power inlets and distribution centers, especially because sharing power lines with other departments or companies may not be advisable. Circuit breakers should be easily accessible to embryologists or building maintenance staff. General knowledge of the mechanical and electrical engineering of the building and the laboratory specifically will always be advantageous. Leaving all the building mechanics and facilities to other individuals is often counterproductive. Embryologists need to be involved with facilities management and be updated with construction decisions inside and outside the building in a timely manner.

Ample storage spaces should always be planned for IVF laboratories. In the absence of dedicated storage space, laboratory space ends up being used instead, filling all cabinets and negating any advantages of the original design. The dedicated storage area should be used to stock all materials in sufficient quantity to maintain a steady supply. A further reason to include storage areas in laboratory design—sufficient on its own to justify the space—is that new supplies, including sterile disposable items, release multiple compounds for prolonged periods. This “out-gassing” has been determined to be a major cause of air pollution in a number of laboratories in which supplies were stored inside the lab. Separate storage space therefore provides the best chance of good air quality, especially when it is supplied by separate air handling system. It should be large enough to handle bulky items as well as mobile shelving for boxes. One should be careful to avoid the natural inclination to save extra trips by bringing too many items into the laboratory, or the gains made by careful design may be lost. As a possible makeshift solution, storage cabinetry in the laboratory can be designed with separate negative pressure air handling in order to minimize release of VOCs from off-gassing package materials.

**MICROSCOPES AND VISUALIZATION OF CELLS**

Though dissecting microscopes are crucial for the general handling of gametes and embryos, many people still consider inverted microscopes to be a luxury even though they are in regular use with micromanipulation systems. Proper visualization of embryos is key to successful embryo selection for transfer or freezing; if the equipment is first class, visualization can be done quickly and accurately (16). Even so, appropriately detailed assessment is still dependent on the use of an oil overlay system to prevent damage by prolonged exposure. Each workstation and microscope should be equipped with a still camera and/or video camera and monitor. Still photos can be placed in the patient file and video footage permits speedy review of embryonic features with colleagues after the gametes are safely returned to the incubator; this is also helpful for training of new embryologists. Interference optics such as Hoffman and Nomarski are preferable because they permit the best measure of detail and depth. Novel visualization of internal elements such as spindles using polarized microscopy requires additional equipment, but can be incorporated into routine operation (17). Ideally, the captured photos should be digitally stored for recall in the clinic’s medical database.

Development of new time-lapse microscopy technologies has made continuous and uninterrupted monitoring of embryo development a reality. This is an invaluable
teaching and learning tool. However, equipment costs are high and, for many laboratories, prohibitive. Equipment for time-lapse technology can be sizable and may require separate consideration in terms of lab design and bench space.

CONSTRUCTION, RENOVATION, AND BUILDING MATERIALS

Construction and renovation can introduce a variety of compounds into the environment of the ART laboratory, either temporarily or permanently. Either can have major adverse effects on the outcome of operations (10–12,18,19). The impact of the exterior environment on IVF success has been demonstrated. Pollutants can have a significant negative effect on success in an IVF laboratory (10,20). These effects can range from delayed or abnormal embryonic development, reduced or failed fertilization, and reduced implantation rates to pregnancy loss and failure of a treatment cycle. Many of the damaging materials are organic chemicals that are released or out-gassed by paint, adhesives from flooring, cabinets, and general building materials, as well as from laboratory equipment and procedures. It is important to realize that the actual construction phase of the laboratory can cause permanent problems. Furthermore, any subsequent renovation activity in adjacent areas can also cause similar, or even greater problems. Neighboring tenants can be informed of the sensitivity of gametes and embryos in culture. At the very least, changes undertaken in adjacent areas should be supervised by IVF laboratory personnel to minimize potential damage. However, new construction immediately outside the building is considerably more problematic. City works are very hard to predict and nearly impossible to control. A good relationship with the neighbors should be maintained and a working relationship with building owners and city planners should be established so that the IVF laboratory is kept informed of upcoming changes.

For the construction of a new laboratory or if changes are to be made to areas adjacent to the IVF facility, the following guidelines should be followed. First, the area to be demolished and reconstructed needs to be physically isolated from the IVF laboratory (if this is not the new IVF laboratory itself). The degree of isolation should be equivalent to an asbestos or lead abatement project. The isolation should be done through: (1) physical barriers, consisting of poly-sheeting supported by studding where needed; (2) limited access to the construction area and the use of an access passageway with two doors in series; (3) removal of all construction waste via an exterior opening or proper containment of waste before using an interior exit; (4) negative air pressure in the construction area, exhausting to the exterior, far removed from the laboratory’s air intake, and properly located with regard to the prevailing winds and exterior airflow; (5) extra interior fans during any painting or the use of adhesives to maximize removal of noxious fumes; and (6) compiling and logging of Material Safety Data Sheets (MSDS) for all paints, solvents, and adhesives in use.

Follow-up investigations with manufacturers and their representatives may be helpful because specifications of equipment may be changed without notice. The negative pressurization of the laboratory space requires continuous visual confirmation via a ball and tube pressure indicator or simply paper strips. Periodic sampling for particulates, aldehydes, and organics could be done outside the demolition and construction site, provided this is economically feasible. Alternatively, tracer gas studies can be done to verify containment. The general contractor of the demolition and construction should be briefed in detail on the need to protect the IVF facility and techniques to accomplish this. When possible, the actual members of the construction crew themselves should be selected and briefed in detail. Large filter units using filter pellets of carbon and permanganate can be placed strategically. Uptake of organics can be assayed, but the frequency of routine filter changes should be increased during periods of construction activity.

SELECTION OF BUILDING MATERIALS

Many materials release significant amounts of VOCs and a typical list includes paints, adhesives, glues, sealants, and caulking, which release alkanes, aromatics, alcohols, aldehydes, ketones, and other classes of organic materials. This section outlines steps to be taken in order to reduce these out-gassing chemicals. Any and all interior painting throughout the facility should only be done on prepared surfaces with water-based paint formulated for low VOC potential. During any painting, auxiliary ventilation should be provided using large industrial construction fans, with exhaust vented to the exterior. Paints that can significantly influence air quality should be emission tested (some suppliers already have these test results available). Safety Data Sheets (SDS; previously MSDS) are generally available for construction materials. Suppliers should be encouraged to conduct product testing for emission potential. The variety of materials and applications complicates the testing process, but several procedures have been developed to identify and quantify the compounds released by building materials and furnishings. Interior paints must be water-based, low-volatile paints with acrylic, vinyl acrylic, alkyd, or acrylic latex polymers. Paints meeting this specification can also contain certain inorganic materials. Low-volatile paints may still contain low concentrations of certain organics. No interior paint should contain formaldehyde, acetaldehyde, isocyanates, reactive amines, phenols, and other water-soluble volatile organics. Adhesive glues, sealants, and caulking materials present some of the same problems as paints. None of these materials used in the interior should contain formaldehyde, benzaldehyde, phenol, and similar substances. Although water-based versions of these are generally not available, their composition varies widely. Silicone materials are preferred whenever possible, particularly for sealants and caulking work. A complete list of guidelines for material use during the construction of a tissue culture laboratory is available elsewhere (21).
“BURNING IN” OF THE FINISHED FACILITY

New IVF laboratories and new facilities around existing laboratories have often been plagued by complaints of occupants who experience discomfort from the chemicals released by new construction and furnishings. The ambient levels of many of these materials can be reduced by “burning in” the facility. A typical burn-in consists of increasing the temperature of the new area by 10°C–20°C and increasing the ventilation rate; even higher temperatures are acceptable. The combination of elevated temperature and higher air exchange aids in the removal of the volatile organics. Upon completion of the construction, the air handling system should be properly configured for the burn-in of the newly constructed area. As previously stated, the system must be capable of supplying the space with air at a temperature of 30°C–35°C, at less than 40% relative humidity. The burn-in period can range from 10 to 28 days, and the IVF laboratory should be kept closed during this time. If these temperatures cannot be reached by the base system, use auxiliary electrical heating to reach the minimum temperature. During burn-in, all lighting and some auxiliary equipment should be turned on and left running continuously. Naturally, ventilation is critical if redistribution of irritants is to be avoided; the whole purpose is to purge the air repeatedly. Auxiliary equipment should of course be monitored during the burn-in.

The same burn-in principle applies to newly purchased incubators or other laboratory equipment. Removal of volatile organics is especially important in the critical microenvironment of the incubator. Whenever possible, it is advantageous to purchase incubators months in advance of their intended initial use and to operate them at an elevated temperature in a clean, protected location. An existing embryology laboratory is not a good space for the burn-in of a new incubator.

Most of the equipment available for use in an ART laboratory has not been designed or manufactured to be VOC-free. Special attention must be invested in new laboratory equipment to eliminate or reduce VOC levels by as much as possible before first use.

Most manufacturers do not address the issues of VOC out-gassing in product manuals, even if the equipment has been expressly designed for the IVF field. Unpacking, cleaning, and operating equipment prior to final installation in a lab for out-gassing the “new car smell” is always recommended.

Incubators should be unpacked, inspected, cleaned, out-gassed, operated, re-cleaned, calibrated, and tested well in advance. The process can take several months to accomplish, but is generally a very essential task that is rewarded with the most suitable culture system that the selected incubator model can provide. When possible, operating incubators at elevated temperatures above the typical culture temperature will hasten the release or burn-off of VOCs. Extended operation at between 40°C–45°C works well to burn off VOCs if this is within the manufacturer’s recommended temperature range. Incubator model VOC loads can vary greatly. Accurate VOC testing may be expensive and time consuming, but it is recommended to test a specific incubator model to determine the new unit’s typical VOC characteristics and how much time out-gassing may require.

Handheld VOC testing devices are available and can be used to help monitor the decline of total VOCs, but cannot match the level of accuracy of an environmental organic chemist’s testing. Handheld VOC meter technology generally is not sensitive enough to monitor low-molecular-weight classes of VOCs. They are reasonably affordable, easily used, and can provide a means of monitoring VOC reduction to help determine if the out-gassing time may be sufficient to observe a reduction of VOCs.

New incubators are generally tested with a mouse embryo assay (MEA), replicating a culture system as part of a new incubator commissioning process. Most laboratories today use some variation of an oil culture system. The oil can serve as an excellent filter against potential VOCs, but may not protect a culture system from the full range of VOC exposure, particularly low-molecular-weight compounds such as aldehydes. Incubator MEA commissioning should include both an oil and an open exposed media test to help evaluate the success of preparing the incubator. The dual MEA approach works well for humidified incubator systems, but may not be applicable if a dry, non-humidified culture system is used. Most dry, non-humidified culture systems are designed to recirculate chamber air and incorporate a VOC filtration strategy. Open culture generally cannot be used with non-humidified incubators. The manufacturer’s recommendations should be followed. Non-humidified incubators may require extended off-gassing and should be tested prior to use in order to confirm that they do not have a VOC issue. Chemical VOC filters should be replaced after burn-off prior to any MEA testing.

Laminar flow hoods and isolettes are also important potential VOC sources that should not be overlooked. They should be given ample time to operate and out-gas as they can contribute to a lab’s VOC contamination load. High-efficiency particulate air (HEPA) and chemical filters should be selected for low-VOC manufacturing traits and also may require off-gassing. Care must be taken when out-gassing laminar flow hoods and isolettes as they require a HEPA-filtered environment or replacement of their filters when transferred to an IVF lab.

After the burn-in is complete, commissioning of the IVF suite should be conducted to verify that the laboratory meets the design specifications. The ventilation and isolation of the laboratory should be verified by a series of tests using basic airflow measurements and tracer gas studies. The particulate levels should be determined to verify that the HEPA system is functional. Particulate sampling can be performed using U.S. Federal Standard 209E. Microbial sampling for aerobic bacteria and fungi is often done in new facilities using an Andersen sampler followed by microbiological culturing and identification. The levels of VOC contamination should be determined. Possible methods are included in the U.S. Environmental
Protection Agency protocols using gas chromatography/mass spectroscopy and high-performance liquid chromatography that is sensitive at the microgram per cubic meter level (22–25).

MAINTENANCE PLANNING AND STERILIZATION
Even the best systems and designs will eventually fail unless they are carefully maintained. The heating, ventilation, and air conditioning (HVAC) will require filter changes, coil cleaning, replacement of drive belts, and chemical purification media. The most prevalent failure concerns the initial particulate filter. These are inexpensive filters designed to keep out large dust particles, plant debris, and insects, among others. If such filters are not replaced promptly and regularly, they will fail, allowing the HVAC unit to become contaminated. The HEPA filters and chemical media also require inspection and periodic replacement. Maintenance staff should report their findings to the IVF laboratory.

The IVF laboratory must have a cleaning facility for surgical instruments. Ongoing use of an autoclave is not a problem as long as the released steam is rapidly exhausted to the outside. This keeps the relative humidity in the facility to controllable limits. Autoclaves should not be placed on the IVF laboratory’s HVAC system, but rather in a room that is built using tight construction and is exhausted directly outside of the building. The use of cold sterilizing agents is not advised. Aldehydes such as glutaraldehyde and ortho-pathaldehyde from the autoclave can be transported inside the IVF laboratory.

INSURANCE ISSUES
ARTs have become common practice worldwide and are regulated by a combination of legislation, regulations, or committee-generated practice standards. The rapid evolution and progress of ART reveal new legal issues that require consideration. Even the patients themselves are changing, as it becomes more acceptable for single women and homosexual couples to seek and receive treatment. Donation of gametes, embryos, and gamete components, enforcement of age limits for treatment, selective fetal reduction, pre-implantation genetic diagnosis, surrogacy, and many other practices in ART present practitioners and society at large with challenges, which are often defined by social norms, religion, and law and are specific to each country.

Furthermore, financial and emotional stresses often burden patients seeking treatment in countries where medicine is not socialized and infertility treatment is not covered by insurance. This translates into an increasing number of ART lawsuits related to failed treatments in spite of generally improved success rates. Laboratory personnel and the laboratory owner should therefore obtain an insurance policy of a sufficiently high level and quality commencing prior to the first day of operations. Litigation-prone issues need special consideration, and include:

- Cancellation of a treatment cycle prior to egg retrieval
- Failure to become pregnant
- Patient identification errors
- Cryostorage mishaps

These issues occur even if experienced practitioners consider themselves at low risk of exposure. Prior to engaging in the practice of ART, protocols must be established to identify potential problem areas and establish countermeasures.

CONCLUSIONS
It may be surprising how many professionals continue to pursue the establishment of new ART clinics at a time when competition is fierce, financial benefits are small, and existing ART services may appear to be approaching saturation in many areas and countries. Appearances can be misleading, however, and ART centers of excellence that deserve the trust and confidence of patients and serve as models for other practices are always needed.

This chapter provides some guidance for those who aspire to establish such outstanding, well thought out and aspired to as models for other practices are always needed.

REFERENCES


22. Seifert B. Regulating indoor air. Presented at the 5th International Conference on Indoor Air Quality and Climate, Toronto, Canada, 1990; 5: 35–49.


New guidelines for setting up an assisted reproduction technology laboratory


Seifert B. Regulating indoor air. Presented at the 5th International Conference on Indoor Air Quality and Climate, Toronto, Canada, 1990; 5: 35B


Quality control Maintaining stability in the laboratory


The assisted reproduction technology laboratory Current standards


Clinical and laboratory guidelines for assisted reproductive technologies in the Nordic Countries: NFOG bulletin supplement. NFOG 1997; 3.


Hartshorne, G.M. and Baker, H., 2006. Fads and foibles in ART; Where is the evidence? Hum Fertil (Camb), 9, 27E.
QAP online FertAid. [Available from: http://www.fertaid.com]
35 Kuhn, A.M. and Youngberg, B.J. , 2002. The need for risk management to evolve to assure a culture of safety. Qual Saf Health Care, 11, 158E.

Evaluation of sperm


Sperm preparation techniques


Assisted reproduction technique using spermatozoa free from HIV-1. AIDS, 20 (7), 967—73.
Chnstie, I.L., Mullen, J.E., Braude, P.R., Rowell, P., Williams, E., Elkington, N., et al., 1998. Assisted conception in HIV discordant couples wishing to have children. AIDS, 14 (11), 1611B.
Pasquier, C., Daudin, M., Righi, L., et al., 2000. Sperm washing and virus nucleic acid detection to reduce HIV and hepatitis C virus transmission in serodiscordant couples wishing to have children. AIDS, 14 (12), 2093B.
Pasquier, C., Daudin, M., Righi, L., et al., 2000. Sperm washing and virus nucleic acid detection to reduce HIV and hepatitis C virus transmission in serodiscordant couples wishing to have children. AIDS, 14 (12), 2093B.
Fiore, J.R., Lorusso, F., Vaccar, M., LaMida, N., Greco, P. and De Palo, R., 2005. The efficiency of sperm washing in removing human immunodeficiency virus type 1 varies according to the seminal viral load. Fertil Steril, 84 (1), 235B.


Sperm chromatin assessment


methyl and ethyl methanesulfonate (MMS and EMS). Mut□□t Res, 198, 131□□□44.
Matsuda, Y. and Tobari, I., 1988. Chromosomal analysis in mouse eggs fertilized in vitro with sperm exposed to ultraviolet light (UV) and methyl and ethyl methanesulfonate (MMS and EMS). Mut□□t Res, 198, 131□□□44.
Bench, G.S., Friz, A.M., Corzett, M.H., et al., 1996. DNA and total protamine masses in individual sperm from fertile mammalian subjects. Cytometry, 23, 263□□□44.


Muriel, L., Goyanes, V., Segrelles, E., et al., 2007. Increased aneuploidy rate in sperm with fragmented DNA as determined by the sperm chromatin dispersion (SCD) test and FISH analysis. J Androl, 28, 38B.


Gosálvez, J., Rodríguez-Predreira, M., Mosquera, A., et al., 2014. Characterisation of a subpopulation of sperm with massive nuclear damage, as recognised with the sperm chromatin dispersion test. Andrologia, 46, 602B.


Meseguer, M., Santiso, R., Garrido, N., et al., 2009. Sperm DNA fragmentation levels in testicular sperm samples from azoospermic males as assessed by the sperm chromatin dispersion (SCD) test and FISH analysis. J Androl, 28, 38B.


Reprod Biomed Online, 8, 616–27.


Irez, T., Ocal, P., Guralp, O., et al., 2011. Different serum anti-Mullerian hormone concentrations are associated with oocyte quality, embryo characteristics and IVF-ICSI outcomes. Arch Gynecol Obstet, 284, 1209–1215.


Colamaria S. Female age, number of mature eggs and biopsied blastocysts effectively define the chance for obtaining at least one euploid embryo: Implication for counselling and decision-making during blastocyst stage preimplantation genetic screening cycles. Oral Presentation at the ESHRE 2015 Annual Meeting. Lisbon, Portugal, Session 35, June 16, 2015.


Fatehi, A.N., Roelen, B.A., Colenbrander, B., et al., 2005. Presence of cumulus cells during in vitro fertilization protects the bovine oocyte against oxidative stress and improves first cleavage but does not affect further development. Zygote, 13, 177B.


De Vos, A., Van de Velde, H., Joris, H., et al., 1999. In-vitro matured metaphase-I oocytes have a lower fertilization rate but similar embryo quality as mature metaphase-II oocytes after intracytoplasmic sperm injection. Hum Reprod, 14, 1859B.


Pickering, S.J., Braude, P.R., Johnson, M.H., et al., 1990. Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte. Fertil Steril, 54, 102B.


Volgelock, K., Shean, L., Goldfarb, J., et al., 1998. The meiotic competence of in-vitro matured human oocytes is influenced by donor age: Evidence that folliculogenesis is compromised in the reproductively aged ovary. Hum Reprod, 13, 1540B.


Moon, J.H., Hyun, C.S., Lee, S.W., et al., 2003. Visualization of the metaphase II meiotic spindle in living human oocytes using the PolScope enables the prediction of embryonic developmental competence after ICSI. Hum Reprod, 18, 817B.
Relationship between meiotic spindle location with regard to the polar body position and oocyte developmental potential after ICSI. Hum Reprod, 18, 1289-1293.

Meiotic spindle location and identification and its effect on embryonic cleavage plane and early development. Hum Reprod, 18, 239-245.


Zona pellucida birefringence score and meiotic spindle visualization in relation to embryo development and ICSI outcomes. Reprod Biomed Online, 18, 681-688.

Tracking of oocyte dysmorphisms for ICSI patients may prove relevant to the developmental potential. Hum Reprod, 16, 2118-2123.

Tracking of oocyte dysmorphisms for ICSI patients may prove relevant to the developmental potential. Hum Reprod, 12, 1267-1270.

Oocyte zona pellucida and meiotic spindle birefringence as a biomarker of pregnancy rate outcome in IIF-ICSI treatment. Ginekol Pol, 85, 264-270.

105 Korkmaz, C., Sakinci, M., Bayoglu Tekin, Y., et al., 2014. Do quantitative birefringence characteristics of meiotic spindle and zona pellucida have an impact on implantation in single embryo transfer cycles? Arch Gynecol Obstet, 289, 433-439.


Effect of oocyte morphology on embryo development and implantation. Reprod Biomed Online, 12, 608-612.

Elective transfer of embryos selected on the basis of first polar body morphology is not related to embryo quality after intracytoplasmic sperm injection. Hum Reprod, 15, 4273-4278.


Is there any predictive value of first polar body morphology for embryo genotype or developmental potential? Reprod Biomed Online, 7, 336-341.


The effect of selecting oocytes for insemination and transferring all resultant embryos without selection on outcomes of assisted. Fertil Steril, 91, 96-100.

Oocyte morphology predicts outcome of intracytoplasmic sperm injection. Hum Reprod, 12, 1267-1270.

Elective transfer of embryos selected on the basis of first polar body morphology is associated with increased rates of implantation and pregnancy. Fertil Steril, 72, 599-603.

First polar body morphology before ICSI is not related to embryo quality or pregnancy rate. Hum Reprod, 19, 2334-2340.

Significance of metaphase II human oocyte morphology on ICSI outcome. Fertil Steril, 90, 1692-1696.

First polar body morphology and blastocyst formation rate in ICSI patients. Hum Reprod, 17, 2415-2421.

Correlation between first polar body morphology and further embryo development. Acta Biol Hung, 57, 331-342.


Is there any predictive value of first polar body morphology for embryo genotype or developmental potential? Reprod Biomed Online, 7, 336-341.


Aptosis in human cumulus cells in relation to zona pellucida thickness variation, maturation stage, and cleavage of the corresponding oocyte after intracytoplasmic sperm injection. Fertil Steril, 77, 511-516.

High magnitude of light retardation by the zona pellucida is associated with conception cycles. Hum Reprod, 20, 1596-1596.

Oocyte morphology predicts outcome of intracytoplasmic sperm injection. Hum Reprod, 12, 1267-1267.

Tracking of oocyte dysmorphisms for ICSI patients may prove relevant to the outcome in subsequent patient cycles. Hum Reprod, 16, 2118-2118.


Tesarik, J. and Greco, E., 1999. The probability of abnormal pre-implantation development can be predicted by a single static observation on pronuclear stage morphology. Hum Reprod, 14, 1318-1322.


Preparation and evaluation of oocytes for intracytoplasmic sperm injection


De Sutter, P., Dozortsev, D., Qian, C. and Dhont, M., 1996. Oocyte morphology does not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection. Hum Reprod, 11, 595.


Advanced sperm selection techniques for intracytoplasmic sperm injection


Majumdar, G. and Majumdar, A., 2013. A prospective randomized study to evaluate the effect of hyaluronic acid sperm selection on the intracytoplasmic sperm injection outcome of patients with unexplained infertility having normal semen parameters. J Assist Reprod Genet, 30 (11), 1471-2B.


Use of in vitro maturation in a clinical setting: Patient populations and outcomes


Dowling-Lacey, D., Jones, E., Bocca, S., et al., 2010. Two singleton live birth after the transfer of cryopreserved-thawed day-3 embryos following an unstimulated in-vitro oocyte maturation cycle. Reprod Biomed Online, 20, 387B.


de Araujo, C.H., Nogueira, D., de Araujo, M.C., et al., 2009. Supplemented tissue culture medium 199 is a better medium for in vitro maturation of oocytes from women with polycystic ovary syndrome than human tubal fluid. Fertil Steril, 91, 509B.


Cha, K.Y., Han, S.Y., Chung, H.M., et al., 2000. Pregnancies and deliveries after in vitro maturation culture followed by in vitro fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. Fertil Steril, 73, 978B.


Ehrmann, D.A., Cavaghan, M.K., Imperial, J., et al., 1997. Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome. J Clin Endocrinol Metab, 82, 524D.

Morin-Papunen, L.C., Koivunen, R.M., Ruokonen, A., et al., 1998. Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. Fertil Steril, 69, 691E.


139 Boomsma, C.M., Eijkemans, M.J., Hughes, E.G., et al., 2006. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update, 12, 673B.


Palomba, S., Russo, T., Orio, F., Jr., et al., 2006. Uterine effects of metformin administration in anovulatory cycle women with polycystic ovary syndrome. Hum Reprod, 21, 457B.


Fadini, R., Miglini Renzini, M., Guamieri, T., et al., 2012. Comparison of the obstetric and perinatal outcomes of children conceived from in vitro maturation treatments with birth from conventional ICSI cycles. Human Reprod, 27, 3601B.


Basatemur, E. and Sutcliffe, A., 2008. Follow-up of children born after ART. Placenta, 29 (Suppl B), 1353D-1359D.


Omelet, W., Peerka, K., De Sutter, P., et al., 2005. Perinatal outcome of ICSI pregnancies compared with a matched group of natural conception pregnancies in Flanders (Belgium): A cohort study. Reprod Biomed Online, 11, 244-252.


Gicquel, C., Gaston, V., Mandelbaum, J., et al., 2003. In vitro fertilization may increase the risk of Beckwith-Wiedemann syndrome related to the abnormal imprinting of the KCN1OT gene. Am J Hum Genet, 72, 1338D-1339D.
Assisted hatching


Hiraoka, K., Fuchiwaki, M., Horaoka, K., et al., 2008. Effect of the size of zona pellucida outcome of frozen cleaved embryos that were cultured to blastocyst after thawing in women with multiple implantation failures of embryo transfer: A retrospective study. J Assist Reprod Genet, 25, 129.


**Human embryo biopsy procedures**


Tobler, K., et al., 2014. The potential use of blastocyst fluid (BF) from expanded blastocysts as a less invasive form of embryo biopsy for preimplantation genetic testing. Fertil Steril, 3 (102), e183-e188.


**Assisted oocyte activation Current understanding, practice, and future perspectives**


Heytens, E., Parrington, J., Coward, K., et al., 2009. Reduced amounts and abnormal forms of phospholipase C zeta in spermatozoa from infertile men. Hum Reprod, 24, 2417B.


Yanagida, K., 2004. Complete fertilization failure in ICSI. Hum Cell, 17, 187B.


Heindryckx, B., Van der Elst, J., De Sutter, P. and Dhont, M., 2005. Treatment option for sperm- and oocyte-related fertilization failure: Assisted oocyte activation following diagnostic heterologous ICSI. Hum Reprod, 20, 22376.


Vanden Meerschaut, F., Nikiforaki, D., De Roo, C., Lierman, S., Qian, C., Schmitt-John, T., De Sutter, P. and Heindryckx, B., 2013. Comparison of pre- and post-implantation development following the application of three artificial activating stimuli in a mouse model with round-headed sperm cells deficient for oocyte activation. Hum Reprod, 28, 11906.


Analysis of fertilization


Tesarik, J. and Greco, E. , 1999. The probability of abnormal pre-implantation development can be predicted by a single static observation on pronuclear stage morphology. Hum Reprod, 14, 1318.
Reprod Biomed Online, 7, 86□□□90.


Gardner, D.K. , Lane M. The 2-cell block in CF1 mouse embryos is associated with an increase in glycolysis and a decrease in tricarboxylic acid (TCA) cycle activity: Alleviation of the 2-cell block is associated with the restoration of in vivo metabolic pathway activities. Biol Reprod 1993; 49(Suppl 1): 152 (Abstract).


Takahashi Y , First N.L. In vitro development of bovine one-cell embryos influence of glucose, lactate, amino acids and vitamins.

Therioenoloy 1992; 37: 963-78.


Gardner, D.K. and Lane, M. , 1996. Allelization of the B-cell block and development to the blastocyst of CF1 mouse embryos: Role of amino acids. EDTA and physical parameters. Hum Reprod, 11, 2703D.


Lee YS, Thouas GA, Gardner DK. Developmental kinetics of cleavage stage mouse embryos are related to their subsequent carbohydrate and amino acid utilization at the blastocyst stage. Hum Reprod. 2015;30(3):543–50.


Evaluation of embryo quality: Time-lapse imaging to assess embryo morphokinetics


---

**Evaluation of embryo quality: Proteomic strategies**


Brinster RL. Protein content of the mouse embryo during the first five days of development. J Reprod Fertil. 1967;13:413-9.


Bianchi V, Coticchio G, Distrais V, et al. Differential sucrose concentration during dehydration (0.2 mol/L) and rehydration (0.3 mol/L) increases the implantation rate of frozen human oocytes. Reprod Biomed Online. 2007;14:64-72.
Gook DA, Osborn SM, Johnston WI. Cryopreservation of mouse and human oocytes using 1,2-propanediol and the configuration of the meiotic spindle. Hum Reprod. 1993;8:1101-1107.
Lassalle B, Testart J, Renard JP. Human embryo features that influence the success of cryopreservation with the use of 1,2 propanediol. Fertil Steril. 1985;44:645-651.


Chen SU, Lien YR, Tsai YY, Chang LJ, Ho HN, Yang YS. Successful pregnancy occurred from slowly freezing human oocytes using the regime of 1.5 mol/L 1,2-propanediol with 0.3 mol/L sucrose. Hum Reprod. 2002;17:1412□□□2.


Bianchi V, Lappi M, Bonu MA, Borini A. Oocyte slow freezing using a 0.2-0.3 M sucrose concentration protocol: Is it really the time to trash the cryopreservation method? Fertil Steril. 2012;97:1101□□□1.


The human oocyte: Vitrification Vitrification


The human embryo: Slow freezing


Lane M, Maybach JM, Hooper K, Hasler JF, Gardner DK. Cryo-survival and development of bovine blastocysts are enhanced by culture with recombinant albumin and hyaluronan. Mol Reprod Dev. 2003;64:70-76.


The human embryo: Vitrification

Vitrification


308 Rall WF , Meyer TK . Zona fracture damage and its avoidance during the cryopreservation of mammalian embryos. Theriogenology. 1989;31(3):683

Chang CC , et al. The oocyte spindle is preserved by 1,2-propanediol during slow freezing. Fertil Steril. 2010;93(5):1430
Vicente JS , Garcia-Ximenez F . Osmotic and cryoprotectant effects of a mixture of DMSO and ethylene glycol on rabbit morulae. Theriogenology. 1994;42(7):1205
Kuleshova LL , et al. Sugars exert a major influence on the vitrification properties of ethylene glycol-based solutions and have low toxicity to embryos and oocytes. Cryobiology. 1999;38(2):119
Kuleshova LL , Shaw JM , Trounson AO . Studies on replacing most of the penetrating cryoprotectant by polymers for embryo cryopreservation. Cryobiology. 2001;43(1):21
Rall WF . Factors affecting the survival of mouse embryos cryopreserved by vitrification. Cryobiology. 1987;24(5):387
Lane M , et al. Cryo-survival and development of bovine blastocysts are enhanced by culture with recombinant alburnin and hyaluronan. Mol Reprod Dev. 2003;64(1):70
Eto TK , Rubinsky B . Antifreeze glycoproteins increase solution viscosity. Biochem Biophys Res Commun. 1993;197(2):927


El-Danasouri I, Selman H. Successful pregnancies and deliveries after a simple vitrification protocol for day 3 human embryos. Fertil Steril. 2001;76(2):400E.


Mazur P, Seki S. Survival of mouse oocytes after being cooled in a vitrification solution to -196 degrees C at 95 degrees to 70,000 degrees C/min and warmed at 610 degrees to 118,000 degrees C/min: A new paradigm for cryopreservation by vitriifica- tion. Cryobiology. 2011;62(1):1–7.


Managing an oocyte bank


Severe male factor infertility: Genetic consequences and recommendations for genetic testing


Polar body biopsy and its clinical application


Preimplantation genetic diagnosis for infertility


Genetic analysis of the embryo


Ray PF, Handsyde AH. Increasing the denaturation temperature during the first cycles of amplification reduces allele dropout from single cells for preimplantation genetic diagnosis. Mol Hum Reprod. 1996;2:213G.


Ferre F. Quantitative or semi-quantitative PCR: Reality versus myth. PCR Methods Appl. 1992;2:1B.


Cheung VG, Nelson SF. Whole genome amplification using a degenerate oligonucleotide primer allows hundreds of genotypes to be performed on less than one nanogram of genomic DNA. Proc Natl Acad Sci USA. 1996;93:14676G.


Ferre F. Quantitative or semi-quantitative PCR: Reality versus myth. PCR Methods Appl. 1992;2:1B.


Diagnosis of endometrial receptivity and the embryo-endometrial dialog


Artificial gametes The oocyte


Hayashi K, Ohta H, Kurimoto K, Aramaki S, Saijou M. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cell. Stem. 2011;14:519B.


Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663B.


Grieve KM, McLaughlin M, Dunlop CE, Telfer EE, Anderson RA. The controversial existence and functional potential of oogonial stem cells (OSCs). Maturitas. 2015;82:278B.


Hernandez SF, Vahidi NA, Park S, Weitzel RP, Tisdale J, Rueda BR, Wolff EF. Characterization of extracellular DDX4-or Ddx4-positive ovarian cells. Maturitas. 2015;82:278B.


Telfer EE, McLaughlin M, Ding C, Thong KJ. A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin. Hum Reprod. 2008;23:1151B.
Microfluidics in assisted reproduction technology: Towards automation of the in vitro fertilization laboratory


McLaughlin M, Telfer EE. Oocyte development in bovine primordial follicles is promoted by activin and FSH within a two-step serum-free culture system. Reproduction. 2010;139:97.


Ting AY, Yeoman RR, Lawson MS, Zelinski MB. In vitro development of secondary follicles from cryopreserved rhesus macaque ovarian tissue after slow-rate freeze or vitrification. Hum Reprod. 2011;26:2461.


doi: 10.1038/nature2014.


Quality management in reproductive medicine


The environment and reproduction


Trubo R . Endocrine-disrupting chemicals probed as potential pathways to illness. JAMA . 2005;294:2918


Richthoff J, Rylander L, Jonsson BA, et al. Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population. Environ Health Perspect. 2003;111:994-1006.


NieHS: Bisphenol A (BPA) Questions and Answers about Bisphenol A. http://www.niehs.nih.gov/health-topics/agents/sya-bpa/index.cfm


https://www.dhs.wisconsin.gov/environmental/pcb-fish.htm

Richthoff J, Rylander L, Jonsson BA, et al. Serum levels of 2,2'-4,4',5,5'-hexachlorobiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population. Environ Health Perspect. 2003;111:40910.

Davidson PW, Myers GJ, Weiss B, Shamlaye CF, Cox C. Prenatal methyl mercury exposure from fish consumption and child
Hibbeln JR, Davis JM, Steer C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood
Walsh PM, Wilkins BL, Crowe SR, et al. Polychlorinated biphenyls in human follicular fluid and maternal serum of women from
Younglai EV, Foster WG, Hughes EG, Trim K, Jarrell JF. Levels of environmental contaminants in human follicular fluid, serum, and
These results indicate that the exposure to polychlorinated biphenyls and other environmental pollutants can have a significant impact on reproductive outcomes, including decreased pregnancy rates and potential adverse effects on the health of the offspring. Further research is needed to fully understand the mechanisms underlying these observations and to develop strategies for reducing exposure to these harmful substances.
Indications for in vitro fertilization treatment From diagnosis to prognosis


http://www.epa.gov/laws-regulations/summary- toxic-substances-control-act


http://www.ewg.org/foodnews/summary/


http://www.epa.gov/laws-regulations/summary- toxic-substances-control-act


Dor J, Seidman DS, Ben-Shlomo I, Levran D, Ben-Rafael Z, Mashiach S. Cumulative pregnancy rate following in-vitro fertilization: The significance of age and infertility aetiology. Hum Reprod. 1996;11:425B.


Aboulghar MA, Mansour RT, Serour GI. Controversies in the modern management of hydrosalpinx. Hum Reprod Update. 1999;4:882B.


Dor J, Shulman A, Levran D, Ben-Rafael Z, Rudak E, Mashiach S. The treatment of patients with polycystic ovarian syndrome by in-vitro fertilization and embryo transfer: A comparison of results with those of patients with tubal infertility. Hum Reprod. 1999;5:816B.


Schlegel PN, Girardi SK. Clinical review 87: In vitro fertilization for male factor infertility. J Clin Endocrinol Metab. 1997;82:709B.


Devroyo P, Vandervorst M, Nagy P. Van Steirteghem Do we treat the male or his gamete? Hum Reprod. 1998;13(Suppl 1):178B.


Verpoest W, Tournaye H. ICSI: Hype or hazard? Hum Fertil (Camb). 2006;9:81B.


Collins JA, Rowe TC. Age of the female partner is a prognostic factor in prolonged unexplained infertility: A multicenter study. Fertil Steril.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple.


Hull MG, Fleming CF, Hughes AO, McDermott A. The age-related decline in female fecundity: A quantitative controlled study of implanting capacity and survival of individual embryos after in vitro fertilization. Fertil Steril. 1996;65:783E.


Chuang CC, Chen CD, Chao KH, Chen SU, Ho CN, Yang YS. Age is a better predictor of pregnancy potential than basal follicle-stimulating hormone levels in women undergoing in vitro fertilization. Fertil Steril. 2003;79:63E.


te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update. 2002;8:141E.


Initial investigation of the infertile couple


Kazer RR. Endometrial biopsy should be abandoned as a routine component of the infertility evaluation. Fertil Steril 2004; 82(5): 1297; discussion 3008.


Prognostic testing for ovarian reserve


Hagen CP, Vestergaard S, Juul A, et al. Low concentration of circulating antim□□llerian hormone is not predictive of reduced fecundability.
Steiner AZ, Herring AH, Kesner JS, Meadows JW, Stanczyk FZ, Hoberman S, Baird DD. Antim□□llerian hormone as a predictor of.
Lawson R, El Toukhy T, Kassab A, et al. Poor response to ovulation induction is a stronger predictor of early menopause than elevated 
Seifer DB, MacLaughlin DT, Christie BP, Feng B, Shelden RM. Early follicular serum mullerianinhibiting substance levels are associated with ovarian response during assisted reproductive technique cycles. Fertil Steril. 2002;77:466E71.
Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, Te Velde ER. Expected poor responders on the basis of an antral follicle count 
Lekamge DN, Lane M, Gilchrist RB, Tremellen KP. Increased gonadotrophin stimulation does not improve IVF outcomes in patients with 
Seifer DB, MacLaughlin DT, Christie BP, Feng B, Shelden RM. Early follicular serum mullerianinhibiting substance levels are associated with ovarian response during assisted reproductive technique cycles. Fertil Steril. 2002;77:466E71.
Drugs used for ovarian stimulation
Clomiphene citrate, aromatase inhibitors, metformin, gonadotropins, gonadotropinreleasing hormone analogs, and recombinant gonadotropins


Adashi EY. Clomiphene citrate: Mechanism(s) and site(s) of action hypothesis revisited. Fertil Steril. 1984;42:331-7.


519 Neveu N , Granger L , St-Michel P , Lavoie HB . Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction and achievement of pregnancy in 154 women with polycystic ovary syndrome. Fertil Steril . 2007;87:113□□□4.


Klinikert ER, Broekmans FJ, Looman CW, et al. The antral follicle count is a better marker than basal follicle-stimulating hormone for the selection of older patients with acceptable pregnancy prospects after in vitro fertilization. Fertil Steril. 2005;83:811E.


European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LHand FSH-deficient anovulatory women: A dose-finding study. J Clin Endocrinol Metab. 1998;83:1507E.


Marrs R, Meldrum D, Muasher S, et al. Randomized trial to compare the effect of recombinant human FSH ( follitropin alfa) with or without recombinant human LH in women undergoing assisted reproduction treatment. Reprod Biomed Online. 2004;8:175E.


The role of follicle-stimulating hormone and luteinizing hormone in ovarian stimulation: Current concepts


Endocrine characteristics of assisted reproduction technology cycles


The use of gonadotropin-releasing hormone agonists and the efficiency of in vitro fertilization


Gonadotropin-releasing hormone antagonists in ovarian stimulation for in vitro fertilization


A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone agonist ganirelix (Org 37462) to delay the premature LH surge in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon). The ganirelix dose-finding study group. Hum Reprod 1998; 13(11):3023B.


Borg G, Mannaerts B. Treatment with the gonadotrophin-releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe and convenient: Results of a controlled, randomized, multicentre trial. The European Orgalutran Study Group. Hum Reprod. 2000;15(7):1490B.


559 Griesinger G, Venetis CA, Tarlatzis BC, Kolbianakis EM. To pill or not to pill in GnRH antagonist cycles: The answer is in the data already. Reprod Biomed Online. 2015;31(1):6B.


Gonadotropin-releasing hormone agonist triggering


Gonadotropin-releasing hormone agonist triggering


572 Humaidan P, Westergaard LG, Mikkelsen AL, Fukuda M, Yding Andersen C. Levels of the EGFlike peptide amphiregulin are significantly reduced in follicular fluid after GnRHa triggering of final oocyte maturation. Hum Reprod 2009; 24: O-284.


Kol S. Luteolysis induced by a gonadotropin-releasing hormone agonist is the key to prevention of ovarian hyperstimulation syndrome. Fertil Steril. 2004;81(1):1E.


Humaidan P, Thomassen LH, Alsbjerg B. GnRH trigger when hCG stimulation is modified luteal support with one bolus of hCG should be used with caution in extreme responder patients. Hum Reprod. 2013;28(9):2593E.


Segmentation of in vitro fertilization treatment


Fang C, Huang R, Wei LN, Jia L. Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. Fertil Steril. 2015;103:655B.


Clayton HB, Scheive LA, Peterson HB, Jamieson DJ, Reynolds MA, Wright VC. Ectopic pregnancy risk with assisted reproductive technology procedures. Obstet Gynecol. 2006;107:1034B.

Jun SH, Milki AA. Ectopic pregnancy rates with frozen compared with fresh blastocyst transfer. Fertil Steril. 2007;88:629B.


The use of ovarian reserve biomarkers to tailor ovarian stimulation for in vitro fertilization


Broer SL, Mol BWJ, Hendriks D, Broekmans FJM. The role of antimullerian hormone in prediction of outcome after IVF: Comparison with the antral follicle count. Fertil Steril . 2009;91(3):705."[44]


Nardo LG, Christodoulou D, Gould R, Roberts SA, FitzGerald CT, Laing I. Anti-Mullerian hormone levels and antral follicle count in women enrolled in in vitro fertilization cycles: Relationship to lifestyle factors, chronological age and reproductive history. Gynecol Endocrinol . 2007;23(8):486."[49]


Baker ML, Metcalfe SA, Hutson JM. Serum levels of mullerian inhibiting substance in boys from birth to 18 years, as determined by enzyme immunoassay. J Clin Endocrinol Metab . 1990;70(1):111."[59]


Freeman EW, Sammel MD, Lin H, Gracia CR. Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women. J Clin Endocrinol Metab . 2012;97(5):1873."[64]


Monitoring ovarian response in assisted reproduction (in vitro fertilization and intracytoplasmic sperm injection)


Oocyte collection


---

Oocytes and Success in IVF


---

Oocytes and Pain During Oocyte Recovery


---

Oocytes and Pain During Oocyte Recovery


---

Oocytes and Pain During Oocyte Recovery


Luteal-phase support in assisted reproduction technology


Treatment strategies in assisted reproduction for the poor-responder patient


Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. The antral follicle count is a better marker than basal follicle-stimulating hormone for the selection of older patients with acceptable pregnancy prospects after in vitro fertilization. Fertil Steril. 2005;83:811.


Eldar-Geva T, Margalioth EJ, Ben-Chetrit A, Gal M, Robertson DM, Healy DL, Diamant YZ, Spitz IM. Serum inhibin B levels measured during early FSH stimulation for IVF administration are of value in predicting the number of oocytes to be retrieved in normal and low responders. Hum Reprod. 2002;17:2331.


Hiller SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. Hum Reprod. 1994;9:188.


Lee VC , Chan CC , Ng EH , Yeung WS , Ho PC . Sequential use of letrozole and gonadotrophin in women with poor ovarian reserve: A randomized controlled trial. Reprod Biomed Online . 2011;23:380B.


Recurrent implantation failure

National Center for Chronic Disease Prevention and Health Promotion, Division of Reproductive Health, http://www.cdc.gov/artdata/index.html


Boitrelle F, et al. Large human sperm vacuoles observed in motile spermatozoa under high magnification: Nuclear thumbprints linked to 2011;26:47□□□58.


Ultrasonography in assisted reproduction


Matalliotakis IM , Cakmak H , Mahutte N , Fragouli Y , Acri A , Sakkas D . Women with advanced-stage endometriosis and previous surgery respond less well to gonadotropin stimulation, but have similar IVF implantation and delivery rates compared with women with tubal factor infertility. Fertil Steril . 2007;88:1568□□□2.


Sperm recovery techniques Clinical aspects


Processing and cryopreservation of testicular sperm

Schlegel PN . Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. Hum Reprod . 1999;14(1):131□□□7
Popal W , Nagy ZP . Laboratory processing and intracytoplasmic sperm injection using epididymal and testicular spermatozoa: What can be done to improve outcomes? Clinics (Sao Paulo) . 2013;68(Suppl 1):125□□□8

Embryo transfer technique


Mains I, Van Voorhis BJ. Optimizing the technique of embryo transfer. Fertil Steril. 2010;94(3):785E.


Pointdexter AN 3rd, Thompson DJ, Gibbons WE, Findley WE, Dodson MG, Young RL. Residual embryos in failed embryo transfer. Fertil Steril. 1986;46(2):262E.

Mansour RT, Aboughar MA, Serour GI, Amin YM. Dummy embryo transfer using methylene blue dye. Hum Reprod. 1994;9(7):1257E.


Mansour RT, Aboughar MA. Optimizing the embryo transfer technique. Hum Reprod. 2002;17(5):1149E.

Patton PE, Stoelk EM. Difficult embryo transfer managed with a coaxial catheter system. Fertil Steril. 1993;60(1):182E.


 Eskandar MA, Abou-setta AM, El-Amin M, Almushait MA, Sobande AA. Removal of cervical mucus prior to embryo transfer improves pregnancy rates in women undergoing assisted reproduction. Reprod Biomed Online. 2007;14:308E.


Naaktgeboren N, Broers FC, Heijnsbroek I. Hard to believe hardly discussed, nevertheless very important for the IVF/ICSI result: Embryo transfer technique can double or halve the pregnancy rate. Hum Reprod. 1997;12:149.


Cycle regimes for frozen-thawed embryo transfer


Anesthesia for in vitro fertilization


Barton SE, Misser SA, Berry KF, Ginsburg ES. Female cancer survivors are low responders and have reduced success compared with other patients undergoing assisted reproductive technologies. Fertil Steril. 2012;97:378–381.


Medical considerations of single-embryotransfer


Endometriosis and assisted reproduction technology


Polycystic ovary syndrome and assisted reproduction


MBRRACE-UK. Available from: http://www.npeu.ox.ac.uk/mbrrace-uk/reports


Dewailly D, Catteau-Jonard S, Reyss AC, Maunoury-Lefebvre C, Poncelet E, Pigny P. The excess in 2-5 mm follicles seen at ovarian ultrasonography is tightly associated to the follicular arrest of the polycystic ovary syndrome. Hum Reprod. 2007;22(6):1562.


Piouka A, Farmakiotis D, Katsikis I, Macut D, Gerou S, Pandis D. Anti-Mullerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: Relationship with increased luteinizing hormone levels. Am J Physiol Endocrinol Metab. 2009;296(2):E238.


Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to in-vitro hyperandrogenism, may be the main culprit for the follicular failure. Hum Reprod Update. 2004;10(2):107.


Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Macedo CR. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. Cochrane Database Syst Rev 2014; 11: CD009610.


Management of hydrosalpinx


Chen CD, Yang JH, Lin KC, Chao KH, Ho HN, Yang YS. The significance of cytokines, chemical composition, and murine embryo development in hydrosalpinx fluid for predicting the in vitro fertilization outcome in women with hydrosalpinx. Hum Reprod. 2002;17:128-134.


Ng EH, Chan CC, Tang OS, Chung PC. Comparison of endometrial and subendometrial blood flows among patients with and without hydrosalpinx shown on scanning during in vitro fertilization treatment. Fertil Steril. 2006;85:333-338.


Arora P, Arora RS, Cahill D, Essure® for management of hydrosalpinx prior to in vitro fertilisation—a systematic review and pooled analysis. BJOG. 2014;12:527E.


Fertility preservation strategies


Ting AY, Yeoman RR, Lawson MS, Zelinski MB. In vitro development of secondary follicles from cryopreserved rhesus macaque ovarian tissue after slow-rate freeze or vitrification. Hum Reprod. 2011;26:2461.


Rivkees SA, Crawford JD. The relationship of gonadal activity and chemotherapy-induced gonadal damage. JAMA. 1988;259:2123


van Alphen MMA, van den Kant HJG, de Rooij DG. Repopulation of the seminiferous epithelium of the rhesus monkey after X irradiation. Radiat Res. 1988;113:487


Baukloh V. German Society for Human Reproductive Biology. Retrospective multicentre study on mechanical and enzymatic preparation of fresh and cryopreserved testicular biopsies. Hum Reprod. 2002;17:1788


Brinster RL. Male germ line stem cells: From mice to men. Science. 2007;316:404

Dores C, Alpaugh W, Dobrinski I. From in vitro culture to in vivo models to study testis development and spermatogenesis. Cell Tissue Res. 2012;349:691


Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci USA. 1994;91:11298


Uterus transplantation


Scott JR, Pitkin RM, Yannone ME. Transplantation of the prime uterus. Surg Gynecol Obstet. 1971;133:41B。


Wranning CA, Akhi SN, Diaz-Garcia C. Brannstrom Pregnancy after syngeneic uterus transplantation and spontaneous mating in the rat. Hum Reprod. 2011;26:553B。


Ostensen M. Disease specific problems related to drug therapy in pregnancy. Lupus. 2004;13:746B。

Viral disease and assisted reproduction technology


Severe ovarian hyperstimulation syndrome

Abramov Y, Elchalal U, Schenker JG. An epidemic of severe OHSS; a price we have to pay? Hum Reprod. 1999;14:2181.


Friedlander MA, Loret de Mola JR, Goldfarb JM. Elevated levels of interleukin-6 in ascites and serum from women with ovarian hyperstimulation syndrome. Fertil Steril. 1993;60:826.


La Marca A, Giuliani S, Tirelli A, Bertucci E, Marsella T, Xella S, Volpe A. Anti-Müllerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology. Hum Reprod. 2007;22:766E.

835 Lee TH, Liu CH, Huang CC, Shih YT, Ho HH, Yang YS, Lee MS. Serum anti-Müllerian hormone and estradiol levels as predictors of ovarian hyperstimulation syndrome in assisted reproductive technology cycles. Hum Reprod. 2008;23:160E.


Frydman R, Howles C, Truong F. A double-blind, randomized study to compare recombinant follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin HP) in women undergoing assisted reproductive techniques including intrauterine sperm injection: On behalf of The French Multicentre Trials. Hum Reprod. 2000;15:520E.


Yilmaz N, Uygur D, Özgüz E, Batigolu S. Does coasting, a procedure to avoid ovarian hyperstimulation syndrome, affect subsequent reproduction cycle outcome? Fertil Steril. 2010;94:189E.

Abdalla H, Nicopoulos JDM. The effect of duration of coasting and estradiol drop on the outcome of assisted reproduction: 13 years of experience in 1,068 coasted cycles to prevent ovarian hyperstimulation. Fertil Steril. 2010;94:1757E.


2001;76:1066□□□7.


Louday E, Piazzì A, Engrand P. Results of a phase II, dose finding, clinical study comparing rLH with hCG to induce final follicular maturation prior to IVF. Sixteenth World Congress on Fertility and Sterility, San Francisco, CA, October 4□□□8. (Abs.O-236).


Emperaire JC, Ruffie A. Triggering ovulation with endogenous luteinizing hormone may prevent the ovarian hyperstimulation syndrome. Hum Reprod. 1991;6:506□□□D.


O'Seill KE, Senapati S, Dokras A. Use of gonadotropin-releasing hormone agonist trigger during in vitro fertilization is associated with similar endocrine profiles and oocyte measures in women with and without polycystic ovarian syndrome. Fertil Steril. 2015;103:264□□□D.


Egbage PE. Severe OHSS: how many cases are preventable? Hum Reprod. 2000;15:815-817.


Bleeding, severe pelvic infection, and ectopic pregnancy


SART, ASRM. Assisted reproductive technology in the United States: 201 results generated from the American society for reproductive medicine/society for assisted reproductive technology registry. Fertil Steril 2007; 87: 1253B.


Fang C, Huang R, Wei LN, Jia L. Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. Fertil Steril. 2015;103:655B.


Egg and embryo donation


Gestational surrogacy


Smith VJ. Los Angeles Superior Court, Los Angeles County. 1987: No CF 025653.


The evolving role of the assisted reproduction technology nurse A contemporary review


Rausch DT, Braverman AM. Burnout rates among reproductive endocrinology nurses: The role of personality and infertility attitudes. 56th Annual Meeting of the American Society for Reproductive Medicine. San Diego, CA, October 21-26, 2000, abstract.


Patient support in the assisted reproduction technology program


Covington SN. The role of the mental health professional in reproductive medicine. Fertil Steril. 1995;64:895B.

Blyth E. Guidelines for infertility counselling in different countries: Is there an emerging trend? Hum Reprod. 2012;27:2046B.


Klock SC, Maier D. Guidelines for the provision of psychological evaluations for infertile patients at the University of Connecticut Health Center. Fertil Steril. 1991;56:680B.

Klock SC, Covington SN. Minnesota Multiphasic Personality Inventory (MMPI-2) profiles in the assessment of ovum donors. Fertil Steril. 2010;94:1684B.

Klock SC. Results of the Minnesota Multiphasic Personality Inventory-2 among gestational surrogacy candidates. Int J Gynecol Obstet. 2015;130:257B.

The Practice Committee of the American Society for. Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Recommendations for practices utilizing gestational carriers: A committee opinion. Fertil Steril. 2015;103:e1B.


Boivin J, Scanlan LC, Walker SM. Why are infertile patients not using psychosocial counselling? Hum Reprod. 1999;14:1384B.


Kaklour L, Mackert A. Perceptions of infertility information and support sources among female patients who access the Internet. Fertil Steril. 2011;95:513B.


The relationship between stress and in vitro fertilization outcome


Boivin J, Scianlan LC, Walker SM. Why are infertile patients not using psychosocial counselling? Hum Reprod. 1999;14:1384E.


Religious perspectives on human reproduction


Gad El-Hak AGH. In vitro fertilization and test tube baby. Dar El Iftaa Cairo Egypt. 1980;1225:3213.


Sura Al Bakara 2: 185, Holy Quran.

Sura Al Ahzab 32: 4, Holy Quran.


Genesis 1: 28.

Genesis 30: 14.


Risk and safety management in assisted reproduction technology


