ers.com, for detailed information on obstetrics & gynecology books, visit our website www.jaypeebrothers.com, for detailed information on obstetrics & gynecology book

The Art & Science of Assisted Reproductive Techniques (ART)

Editors
Gautam Nand Allahbadia
Monikaa Malhotra Chawla
Rita Basuray Das
Goral Neeraj Gandhi
Esther Velilla Garcia
Rubina Merchant

2nd Edition

Foreword
Mohamed Aboulghar

JAYPEE

Contents

Introduction

1. **Endocrinology of Assisted Reproductive Techniques** 3
   Zeev Blumenfeld

2. **Modern Work-up of Infertility** 13
   Krishnendu Gupta, Sajal Datta, Bijit Chowdhury

Section 1: Stimulation Strategies

3. **Ovulation Induction with Tamoxifen Citrate** 21
   Padma Rekha Jirge, Umesh Acharya

4. **Defining the Poor Ovarian Response before Controlled Ovarian Hyperstimulation** 27
   Daniel Griffin, David W Schmidt

5. **Aromatase Inhibitors: Their Role in the Treatment of Infertility** 38
   Pankaj Shrivastav, Munira Juzer Furniturewala

6. **Urinary Human FSH versus Recombinant Human FSH** 43
   Eitan Lunenfeld, Tali Silberstein

7. **Stimulation Strategies for Complex IVF Patients** 47
   Franco Lisi, Leonardo Rinaldi

8. **The Dilemma of the Poor Responder: Choices before Oocyte Donation** 55
   Barbara Lawrenz

9. **Programming the Cycle with Oral Contraceptives: Antecedent to the Use of GnRH Antagonists** 61
   Nico Naumann, Hossein G Gholami

10. **GnRH Agonists versus Antagonists: From Physiology to Clinical Success** 64
    Ester Polak de Fried, Fernando Neuspiller, Gerardo Ardiles

11. **Microdose GnRH for the Stimulation of Low Responders** 71
    CAM Jansen, Kathleen E Tucker

12. **Role of GnRH Antagonists in the Management of Poor Responders** 75
    Hossein G Gholami, Nico Naumann, Antonio Barbaro

13. **Alternative Approaches to Ovarian Stimulation and Triggering of Ovulation** 79
    Gautam N Allahbadia, Rubina Merchant, Swati G Allahbadia, Akanksha G Allahbadia

14. **Role of LH in Stimulation Protocols for ART** 107
    Lars Grabow Westergaard, Claus Yding Andersen

15. Role of hMG-HP in Stimulated Cycles for ART 113
Gautam N Allahbadia, Rubina Merchant, Swati G Allahbadia, Akanksha G Allahbadia

16. Protocol for the Good Egg 123
Angeline N Beltsos

17. The Value of Individualized Ovarian Stimulation Protocols 130
Peter Kovacs

18. Luteal Phase Support 140
Valery Mycolayovych Zaporozhan, Vladlena Gennadiivna Dubinina, Ruslan Valentinovich Sobolev

19. Severe OHSS: A Critical Care Physician's Point of View 149
Sanjay C Wagle

20. Protocols for Reducing the OHSS Risk and their Success 152
Monikaa M Chawla, Michael H Fakih, Amal Al-Shunnar

21. Ovulation Induction: A Surgical Approach 159
Attila Vereczkey, Otto Kabdebo, István Szabó

Section 2 : Polycystic Ovary Syndrome

22. Polycystic Ovary Syndrome: An Update 173
Jane M Nani

23. Polycystic Ovary Syndrome: Genetics and Health Consequences 180
Sudip Basu, Nazar N Amso, Jaydip Bhaumik

Section 3: ART Procedures

24. Vaginal Oocyte Retrieval 189
Gautam N Allahbadia, Rubina Merchant, Goral N Gandhi, Akanksha G Allahbadia

25. Gamete Intrafallopian Transfer 200
Andrea Borini, Luca Dal Prato

26. Zygote Intrafallopian Tube Transfer: Patient Selection is the Key to Beneficial Utilization 206
Daniel S Seidman

27. Fallopian Tube Sperm Perfusion 210
Gautam N Allahbadia, Rubina Merchant, Swati G Allahbadia, Akanksha G Allahbadia

28. IVF versus ICSI: How Do You Choose? 217
Akanksha Mishra, Rutvij Dalal

29. Use of Lasers in ART: Clinical Applications and Potential Research Tools 221
Yona Tadir

30. Optimizing Embryo Transfer: Can We Do Better? 229
Asha Reddy

31. Single Embryo Transfer: Possibilities and Limitations 237
Monikaa M Chawla, Michael H Fakih, Amal Al-Shunnar

32. Blastocyst Transfer: One Step Further in the Quest for the Magic Bullet 242
Sarit Avraham, Daniel S Seidman

33. Native Assessment of Sperm Ultramorphology: Clinical Application and Efficacy 245
Rutvij Dalal

Section 4: Laboratory Issues

34. Setting up an ART Laboratory 253
Yousef M Alhelou

35. Semen Analysis for Clinical Interpretation 259
Elizabeth Puscheck, Jay M Bolnick, Rajasingam S Jeyendran

36. Modern Evaluation of Sperm: Clinical Application of the 5th Edition WHO Manual for Processing and Examining Semen 268
Yousef M Alhelou

37. Fundamentals of Sperm Processing Techniques 274
Alan Bolnick, James Craine, Milica Ivanovic, Rajasingam S Jeyendran, Elizabeth Puscheck

38. Sperm Separation Techniques: Comparison and Evaluation of Methods and Gradient Products–Then and Now 280
Kathleen E Tucker, CAM Jansen

39. Prediction of ART Outcome in Male Factor Infertility Patients by a New Semen Quality Score 284
Ashok Agarwal, Rakesh K Sharma

40. Why Should We Assess Oocyte and Embryo Morphology? 298
Kathleen E Tucker, CAM Jansen

41. Embryo Screening: Invasive versus Non-invasive 308
Pere Mir Pardo, Belén Aparicio-Ruiz, Carmen Rubio, Marcos Meseguer, Alex C Varghese

42. Benefits and Drawbacks of Extended Embryo Culture 323
CAM Jansen, P M Rijnders, Kathleen E Tucker

43. Really Just how Important is the Level of Lighting in the IVF Laboratory for Embryo Development? 327
Kathleen E Tucker, CAM Jansen

44. Improving Air Quality in an IVF Laboratory 331
Pratik Tambe, Goral N Gandhi

45. The Mouse Embryo Bioassay: Is it the "Gold Standard" for Quality Control Testing in the IVF Laboratory? 337
Kathleen E Tucker, CAM Jansen

46. *In Vitro* Oocyte Maturation **343**
Ved Prakash Singh

47. *In Vitro* Maturation: Future Clinical Applications **345**
Jin Ho Lim, Weon Young Son, San Hyun Yoon

48. Oxidative Stress and DNA Damage in Human Sperm: The Cleveland Clinic Story **351**
Ashok Agarwal, Aditi Mulgund

49. Quality Management in an Assisted Reproductive Therapy Environment **364**
Malcolm Clarke

50. Quality Assurance and Risk Management in an ART Program **367**
Sheetal Sawankar, Gautam N Allahbadia, Goral N Gandhi, Ritu Hinduja Singh, Sakina Kagalwala

Section 5: Contemporary Thoughts

51. How to Improve Success Rates in IVF **379**
Anjali Malpani, Aniruddha Malpani

52. Current Immunological Assays: Are they Enough to Uncover the Supposed Immune Causes for Assisted Reproduction Failure? **383**
Aygül Demirol, Erdal Aktan, Timur Gürgan

53. Endometriosis and ART **387**
Ved Prakash Singh, Angela Beaten

54. Infertility: Is there Success after Forty? **391**
Daniel B Williams, Anil B Pinto

55. Inheritance of Infertility **397**
Mirudhubashini Govindarajan, MS Lakshmi

56. Ovarian Tissue Preservation in Cancerous Patients: State of the Art **402**
Zeev Blumenfeld

57. Molecular Biology Applied to ART **415**
Silvio Cuneo, Laura Guadarrama, Vanesa Morales, Felipe Caldiño, Sandra Cubillos

58. Women Seeking IVF in Genital Tuberculosis: A Developing Country's Perspective **427**
Jai B Sharma

59. Assisted Reproductive Techniques in HIV Serodiscordant Couples: Practice, Prognosis and Future Prospects **438**
Richard A Ajayi, Nwora A Melie

60. Obstetric Outcomes and Congenital Anomalies after IVF, ICSI and IVM **443**
Barbara Lawrenz

Section 6: Third Party Reproduction

61. Oocyte Donation 451
Siya S Sharma, Sucheta Jindal

62. Oocyte Sharing Programs 455
Gautam N Allahbadia, Rubina Merchant, Goral N Gandhi, Akanksha G Allahbadia

63. Gestational Surrogacy 464
Anil B Pinto, Nona Morgan Swank

64. Germinal Stem Cells: Culture and Replication 471
Jayant G Mehta

65. Cytoplasmic and Nuclear Transfer: New Life for an Old Egg? 479
Hugh C Hensleigh, Samuel S Thatcher

Section 7: Implantation

66. The Endometrium and Implantation 487
Anil B Pinto, Daniel B Williams, Randall R Odem

67. Modulators of Endometrial Receptivity: A Molecular Symphony 493
Rafael C Haciski

68. Endometrial Preparation for Patients Undergoing Frozen-thawed Embryo Transfer Cycles 503
Raoul Orvieto, Benjamin Fisch, Dov Feldberg

69. Pathophysiology of Implantation Failure in IVF 507
Jayant G Mehta

70. Recent Advances in the Understanding of the Immunology of Reproductive Failure 515
Bee K Tan, Alka Prakash

71. Genital Tuberculosis in Recurrent Implantation Failure 524
Jai B Sharma

72. Recurrent Implantation Failure: The Preferred Therapeutic Approach 536
Monikaa M Chawla, Michael H Fakih

73. The Dilemma in Investigating Repeated Pregnancy Loss 543
Asha Baxi, Hemlata Sodhiya

74. Antiphospholipid Antibodies in ART 552
Gautam N Allahbadia, Rubina Merchant, Akanksha G Allahbadia

75. Future Directions in Failed Implantation in ART 568
Suneeta Mittal

Section 8: Cryopreservation

76. Cryopreservation of Oocytes and Embryos 579
Foad Azem, Ben Yosef Dalit, Joseph B Lessing

77. Human Oocyte and Embryo Cryopreservation 584
Matthew D VerMilyea, Joshua Lim, Michael J Tucker

78. Oocyte Vitrification : Principles, Applications and Achievements 595
Goral N Gandhi, Gautam N Allahbadia, Shraddha Shetye-Kapdi, Priyanka Pangerkar, Akanksha G Allahbadia

79. Slow-rate Freezing versus Vitrification for Oocytes and Embryos: An Evaluation of the Methodology and Outcomes 605
Shraddha Shetye-Kapdi, Sakina Kagalwala, Goral N Gandhi, Priyanka Pangerkar

80. Cryopreservation of Human Spermatozoa 614
Frank M Köhn, Wolf Bernhard Schill

81. Cryoprotectant-free Sperm Vitrification for IVF/ICSI: A Novel Technology 619
Sakina Kagalwala, Goral N Gandhi, Shraddha Shetye-Kapdi, Priyanka Pangerkar

82. Can We Make IVF Safer with Vitrification? 625
Goral N Gandhi, Gautam N Allahbadia, Sakina Kagalwala, Shraddha Shetye-Kapdi, Priyanka Pangerkar, Akanksha G Allahbadia

83. Ovarian Cortex Vitrification 630
Pankaj Talwar, Puneet Rana Arora

84. Semen Cryopreservation and Sperm DNA Damage 639
Sakina Kagalwala, Goral N Gandhi, Gautam N Allahbadia, Shraddha Shetye-Kapdi, Priyanka Pangerkar, Akanksha G Allahbadia

Section 9: Endoscopy and ART

85. Fertility Following Laparoscopic Surgery and Hysteroscopic Surgery 649
Ramesh B, Nirmala Sadasivam

86. The Role of Hysteroscopy in the Management of Infertility 657
Carlo De Angelis, Monica Antinori

87. Modern Management of the Ischemic Black-blue Twisted Adnexa 665
Roy Mashiach, Shlomo Mashiach, Daniel S Seidman

88. Fertiloscopy: A New Technique and an Alternative to Conventional Laparoscopy in Infertility 669
Radha Syed

89. Uterine Cavity Evaluation by Hysteroscopy, Saline Infusion Sonohysterography and Transvaginal Sonography Prior to IVF-ICSI 678
Alka Kriplani, Debjyoti Karmakar

90. Hysteroscopic Assessment of Selective Tubal Pressures and Tubal Cannulation by Air Bubble Stents 682
Atul Kumar, Alka Kumar

91. Intrauterine Adhesions 684
Rakesh Sinha

Section 10: Ultrasonography

92. Update on Ultrasound-guided Embryo Transfer 691
Vishvanath C Karande, Sigal Klipstein

93. Cervical Mucus Evaluation by Transvaginal Ultrasonography: A Novel Approach 700
Ran Keidar, Ariel Jaffa, Igal Wolman

Section 11: The Male Factor

94. Non-obstructive Azoospermia: Predictive Criteria for Sperm Retrieval 707
Vijay Kulkarni

95. Epididymal and Testicular Sperm Retrieval 710
Rupin Shah

96. Microdeletions in the Y-Chromosome and Male Infertility 715
Sana N Khan, Rajasingam S Jeyendran, Elizabeth Puscheck

97. Isolated Teratozoospermia 720
Kemal Ozgur, Kevin Coetzee, Roelof Menkveld

98. Hormone Substitution in Male Infertility 730
Frank M Köhn, Wolf Bernhard Schill

Section 12: Preimplantation Genetic Diagnosis

99. Preimplantation Genetic Diagnosis for ART: Why, When and How? 735
Attila Vereczkey, Diána Debreceni, Gyöngyvér Téglás, Marianna Csenki

100. Preimplantation Genetic Screening in Assisted Reproduction Techniques 746
Esther Velilla Garcia, Silvia F Fernández

101. Preimplantation Genetic Diagnosis in Infertile Patients of Advanced Maternal Age 752
Silvia F Fernández, Esther Velilla Garcia

102. Study of the Infertile Men I: Genetic and Non-genetic Causes 756
Ferran Garcia

103. Study of the Infertile Men II: Genetic Studies in Somatic Cells 769
Ferran Garcia

104. Study of the Infertile Men III: Genetic Studies in Germ Cells **778**
Ferran Garcia, Esther Velilla Garcia, Silvia F Fernández

105. Preimplantation Genetic Diagnosis for Single Gene Disorders **784**
Silvia Modamio, Raquel García, Silvia F Fernández

106. Aneuploidy and Time-lapse **793**
Carmen Morales, Silvia F Fernández, Esther Velilla Garcia

107. The Scope of Preimplantation Genetic Diagnosis **799**
Ali M Hellani, Altaf A Kondkar, Khaled K Abu-Amero

Section 13: In Conclusion

108. The Significance of Validating New IVF Technology: Think before You Act **809**
Bryan Woodward

109. Patient Support in the ART Program **815**
Rubina Merchant, Gautam N Allahbadia, Akanksha G Allahbadia

110. Politics, Partnerships and IVF **825**
Sandra K Dill

111. *In Vitro* Fertilization Success from a Clinician's Viewpoint: How to Get it, How to Keep it **831**
Sarah L Keller, Anil B Pinto, Daniel B Williams

112. Bandwagons in IVF Revisited: Does One Size Really Fit All? **839**
Kathleen E Tucker, CAM Jansen

113. Intracytoplasmic Sperm Injection for All? **847**
Jayaprakash Divakaran

114. Legal and Ethical Considerations in Assisted Reproductive Techniques **851**
Ritu Hinduja Singh, Gautam N Allahbadia, Goral N Gandhi, Sheetal Sawankar

Index *857*

SECTION 4

Laboratory Issues

- *Setting up an ART Laboratory*
- *Semen Analysis for Clinical Interpretation*
- *Modern Evaluation of Sperm: Clinical Application of the 5th Edition WHO Manual for Processing and Examining Semen*
- *Fundamentals of Sperm Processing Techniques*
- *Sperm Separation Techniques: Comparison and Evaluation of Methods and Gradient Products—Then and Now*
- *Prediction of ART Outcome in Male Factor Infertility Patients by a New Semen Quality Score*
- *Why Should We Assess Oocyte and Embryo Morphology?*
- *Embryo Screening: Invasive versus Non-invasive*
- *Benefits and Drawbacks of Extended Embryo Culture*
- *Really Just how Important is the Level of Lighting in the IVF Laboratory for Embryo Development?*
- *Improving Air Quality in an IVF Laboratory*
- *The Mouse Embryo Bioassay: Is it the "Gold Standard" for Quality Control Testing in the IVF Laboratory?*
- *In Vitro Oocyte Maturation*
- *In Vitro Maturation: Future Clinical Applications*
- *Oxidative Stress and DNA Damage in Human Sperm: The Cleveland Clinic Story*
- *Quality Management in an Assisted Reproductive Therapy Environment*
- *Quality Assurance and Risk Management in an ART Program*

JAYPEE BROTHERS

34
CHAPTER

Setting up an ART Laboratory

Yousef M Alhelou

INTRODUCTION

The assisted reproductive techniques (ART) laboratory plays the main role in the success of any ART program. All the techniques related to fresh and frozen embryos, oocytes, sperm, oocyte retrieval, surgical sperm extraction, embryo transfer, embryo and oocyte biopsy take place inside this laboratory.

Setting up an ART laboratory depends on the number of expected cycles, the scope of service and the health regulations in the country in which the ART laboratory will be established. However, there are common factors that must met in all the ART laboratories regardless of the size and the service, that is, the laboratory should be safe for the patients, the staff and the gametes and embryos.

CLINICAL DISCUSSION

Feasibility Study

A feasibility study aims to objectively and rationally uncover the strengths and weaknesses of the proposed business, opportunities and threats, as presented by the environment, the resources required to carry the venture through, and ultimately, the prospects for success. In its simplest terms, the two criteria to judge feasibility are cost required and value to be attained. As such, a well-designed feasibility study should provide a historical background of the business, a technical study, marketing research study, legal requirement study and financial study.[1]

Technical Study

The technical study in setting an ART laboratory requires attention to details as missing any part may lead to a long delay in the project. The main parts of the technical study are:

The Building and Installation
This part of the feasibility study deals with the cost of the building, finishing and infrastructure, such as electric installation, calculation of the power required for each room, installation of gases, water installation, air conditions and circulation, water installation and back-up power supply.

Equipment
This includes all the main and supporting equipment, suitability of the equipment for the purpose intended and the scope of service. Depreciation of equipment, service and repair contracts, installation, training, placing the equipment in the correct location. Cost of calibration and daily, weekly, monthly, yearly check where applicable.

Cost of Raw Material
This includes estimation of the cost of raw material such as all types of media, laboratory disposables, operation theater disposables, preoperation and postoperation rooms disposables, chemicals, kits for chemistry, hematology endocrinology and immunology tests, and kits and chemicals for preimplanation genetic diagnosis (PGD).

Staff
This includes the cost of salaries and benefits of all the medical, scientific and administrative staff, the work load distribution and plans to cover regular and emergency leaves, specifying the role and job description of each person.

Cost of Furniture and Office Equipment
This includes the cost of furniture and office equipment such as computer network, software, printers, scanners, telephone centrals, office and medical furniture and cost of depreciation.

Operation Costs
This includes operation costs, such as water and electricity bills, telephone and internet, renewal of laboratory license and accreditation cost, staff licenses renewal, advertisement and external quality control cost.[2,3]

Marketing Study

The marketing study includes the estimation of direct and indirect costs, the cost of each service to the patient, the expected average annual number of *in vitro* fertilization (IVF) cycles and tests, the work capacity of competitors, the points of weakness of competitors, approximate market share,

JAYPEE BROTHERS

the price of similar services offered by competitors, pricing strategy and expected revenue.

Legal Study

The legal study deals with start up fees such as feasibility fees, legal consultation fees, financial fees, administrative fees, subscription fees and bank charges.

It also details license and registration, such as registration in Ministry of Health and related Regulation Authorities, Municipality, Chamber of Commerce, and other parties depending on the country in which the laboratory will be established.

In addition, it studies loan usage (if any) and repayment, and shows how a loan will be spent and what is the refund time and monthly installments.[1,3]

Financial Study

The financial study includes calculation of the investment cost such as property and land, building and installation, main and supporting equipment, office equipment and furniture, start-up cost and working capital. Secondly, calculation of current cost such as depreciation and maintenance of buildings, installations, main and supporting equipment, office equipment and furniture, depreciation of start-up cost and cost of direct and indirect raw materials. Thirdly, calculation of current revenue that includes the expected income from all services. Fourthly, calculation of income statement, which is the current revenues minus the current cost and taxes. And finally, calculating the opening balance sheets, which includes total assets and total capital and liability.[3]

Laboratory Design

Air Quality

In the past, ART units were built in green places, depending on thoughts that environmental factors, such as noise, pollution and stress, may have an effect on the patients and treatment quality. In the present time, most of ART units are constructed inside cities and densely populated areas in order to be accessible to a large population.

The environmental effect should be taken into consideration, as the air quality inside an ART laboratory can be controlled, but cannot be completely isolated from the outside environment or the other parts of the building. When thinking of designing an ART laboratory, the concerned people should make sure that the building and its surroundings will not undergo renovation, demolishing or major changes in the foreseen future. City planning should be evaluated as any activity related to construction and demolishing will jeopardize the pregnancy rate of the planned laboratory. The proposed laboratory should be far from industrial and polluted areas. Air sampling should be done inside and outside the planned laboratory and the level of volatile organic compounds (VOCs) should be measured in order to determine the suitable system to remove the VOCs.[4,5]

Table 34.1 Air quality as defined by the Department of Health in the United Kingdom

Grade	*At rest*		*At operation*	
Particle size	0.5	5	0.5	5
A	3500	0	3500	0
B	3500	0	350000	2000
C	350000	2000	3500000	20000
D	3500000	20000	Not defined	Not defined

Positive air pressure, with 0.1–0.2 inches water and 10–15 air changes (ACH) per hour is a reasonable recommendation to purify the air. The air system should have the ability to control the temperature and humidity all over the year, in addition, it should be equipped with activated charcoal and potassium permanganate filters to adsorb, trap and neutralize the VOCs, and high-efficiency particulate arresting (HEPA) filters to remove dust and microbes. The filters should be regularly inspected and changed according to the manufacturer's instructions or when their efficiency is reduced. A class II safety cabinet should be used for handling embryos and gametes. The Tissue and Cell Directive, issued by the European Union (Directive 2003/9/94/EC), states that tissue and cells must be exposed to air quality of grade A and the background environment should be at least equivalent to Grade D air. The Department of Health in the United Kingdom defines the air quality according to Table 34.1.[4,6]

The laboratory walls and ceiling should have the minimum number of accesses. This generally requires a solid ceiling, sealed lighting and airtight utility connections. Doors need seals and sweeps and should be lockable. Ducts and equipment should be organized in a such a way that preventive and corrective maintenance would be achieved outside the laboratory with minimum disruption.

Air supply equipment should balance outside air with recirculated air, a proportion 70% recirculated and 30% fresh air is appropriate. Air balance can control acceptable levels of VOCs and particles count.

Air outlets and inlets should be carefully spread to avoid flows that can change temperature in specific local areas or expose specific items of equipment to relatively poor air or changes in air quality. A comprehensive arrangement and assessment of all laboratory furniture and equipment is therefore necessary prior to design and installation of air equipment and construction.[4,7]

VOCs can be measured using handheld monitors which use photoionization detectors. The air particle count inside the laboratory, incubators and safety cabinets can be performed by using HandiLaz® Mini, which is available from research instruments company.[4,8]

JAYPEE BROTHERS

Space Design

The well-designed ART laboratory should have four main parts: the Andrology section, where activities related to semen assessment, sperm preparation and surgical sperm retrieval are done, the Embryology section, where oocyte retrieval, fertilization, embryo culture and transfer are performed, the Cryopreservation section in which sperm, oocytes, embryos, ovarian and testicular tissue are frozen or vitrified, and the Micromanipulation section, where intracyoplasmic sperm injection (ICSI), assisted hatching, polar body, embryo and blastocyst biopsy are done.

Appropriate arrangement of equipment, such as incubators, safety cabinets and micromanipulation stations, will reduce the distances that the dishes, tubes and embryo-transfer catheters be moved. Ideally, an embryologist should be able to finish one complete procedure without moving more than five seconds in any direction. This also helps to avoid possible collisions in a busy laboratory. All jobs should be performed under aseptic conditions. There should be separate areas for different kinds of work that are free from distraction and accidents for dedicated, focused and uninterrupted concentration. The flow of work should be according to the sequence of procedures in an (IVF) cycle.

The required number of equipment can be assessed by the expected number of cases and procedure types, and thereby, the number of embryologists expected to work simultaneously. Each embryologist should be given sufficient work space to perform all the procedures without delay.[4,5]

Cryopreservation and storage facilities are often located in separate areas; these should be adjacent to the main laboratory.

It is preferable to prepare semen in a separate laboratory, adjacent to a semen collection room. The semen laboratory should have ample space for microscopes, freezers, centrifuges and a safety cabinet. This room should be at the end of a hallway with its own exit, it should be soundproof and not too large, with a sink and a shower and clear instructions on how to collect semen. The room should be adjacent to the semen preparation laboratory with a double door cupboard type pass through for samples between them. This pass through should have a signalling device so the patient can inform the embryologist that the sample is ready; it also permits the male patients to leave the area without a sample container in their hand.

Administration and paperwork should be performed in separate offices.

Staff change room can be accessed from inside and outside the laboratory.

Building Materials

Most of the building materials release significant amounts of VOCs, including paints, primer paints, adhesives, glues, sealants and caulking. These materials release alkanes, aromatics, aldehydes, ketones, and many other groups of VOCs. All interior painting throughout the ART center should only be done on prepared surfaces with water-based paint, formulated for low VOC release. During any painting process, additional ventilation should be provided using large construction fans, with exhaust vented outside the facility. Paints can significantly influence air quality. The material safety data sheet (MSDS) should be revised before selecting paints type. MSDS are generally available construction materials. It is advisable to perform emission potential test before using the paints. The interior paint must be water-based, low volatile paints with acrylic, vinyl acrylic, alkyl or acrylic latex polymers. No interior paint should contain materials such as formaldehyde, acetaldehyde, benzene, toluene, styrene, xylene and other volatile organic solvents. Adhesive and sealants materials contain some of these VOCs, but water-based materials are not generally available for these applications, although their composition varies widely. Silicone materials are preferred, whenever possible, particularly for sealants work.[4]

Preparing the Laboratory for Work

All new laboratories have chemical release from the building materials and furnishing. The ambient level of VOCs can be reduced by increasing the temperature of the new laboratory by 10–20°C and increasing the ventilation rate. The combination of elevated temperature and higher air exchange aids the removal of VOCs. The air system should be capable of supplying the laboratory with air with a temperature up to 35°C and 50% relative humidity. This preparation time period should be from 20 to 30 days. The laboratory should be kept close during this time and all lighting and equipment should be on and left running the whole time. After this preparation time, the level of VOCs and particles count should be measured to verify acceptable levels. Microbial sampling of aerobic bacteria and fungi should be done followed by microbiological culture and identification.[4]

Equipment

A detailed list of equipment should be prepared and checked against the planned location of each item. It is important to consider the inclusion of extracrucial equipment and spare tools in the laboratory design, to allow for the event of sudden malfunction. It is particularly important to have suitable cryopreservation system, including cryopreservation and storage equipment. A spare incubator should be available, and at least one spare suction device and micromanipulator for micromanipulation should also be included. There are many other items whose malfunction would jeopardize patient care. It may also be necessary to team up with other programs so that the crucial pieces of equipment can be exchanged in case of unexpected failure. Some serious thought is needed when contemplating the number of incubators and incubator

JAYPEE BROTHERS

spaces. The ratio of cases per incubator varies considerably and positively affects the clinical outcome, depending on the number, type, and duration of incubator openings. We recommend one incubator for every 15 cases per month. Several other incubators should be used for general purpose during micromanipulation and other generic purposes in order to further limit the number of incubator openings. A spare incubator is also highly recommended. Strict guidelines must be implemented and adhered to when separating dishes or tubes from patients. Separate compartments should be helpful and can be supplied by certain manufacturers. Servicing and sterilization of incubators should be done according to a schedule and subject to workload. Placement of incubators and other pieces of equipment on large castors is helpful in taking the pieces of equipment outside the laboratory for services and repair. Also, supply and evacuation routes should be planned in advance. A contingency plan should be ready for the cryopreserved samples in events such as fire or earthquake. Liquid nitrogen level alarms, with remote notification capability should be contemplated for all dewars. The route of delivery of liquid nitrogen and other medical gases must be relatively easy, and must be planned in advance.

Liquid nitrogen containers and medical gas cylinders are preferentially placed immediately adjacent to the laboratory in a room with outside access. Pipes and tubes enter the laboratory from this room, and cylinders can be delivered and changed to this room without compromising the laboratory area in any way. This allows the liquid nitrogen to be pumped into the cryopreservation laboratory using minimal lining. Lines should be properly installed and insulated to insure that they do not leak or allow condensation. Stainless steel or copper lines can be used to connect the gas to the laboratory.

Placement of bulky and difficult pieces of equipment should be considered when designing doorways and electrical panels. Emergency generators and uninterrupted power supply (UPS) should always be installed even when power supplies are usually reliable.

Ample storage spaces should always be planned for IVF laboratories. In the absence of dedicated storage spaces, the laboratory space ends up being used instead, filling all cabinets and changing the original design. This storage area should contain all materials in sufficient quantity to maintain a steady supply. A further reason to include storage areas in the laboratory design, is that new supplies, including sterilized disposable items, release multiple compounds for prolonged periods. This release of compounds has been determined to be a major cause of adverse air content in laboratories in which supplies were stored. Separate storage spaces therefore provide the best chance for good air quality, especially when it is supplied by a separate air handling equipment. It should be large enough to handle bulky items as well as mobile shelving for boxes. There should be fire-proof cabinets for flammable chemicals. Carton boxes should not be used in the store as this increases the chance for fire. All items in the store should be placed on shelves or cabinets. No items should be on the floor. A part of the store should be dedicated for spare parts and pieces of equipment.[4,5,7,9,10]

Staffing and Direction

Hands on experience in all aspects of Clinical Embryology are an absolute necessity when starting a new program. The period during which the detailed supervision must continue depends absolutely on the types of skills required and the daily case load and the total time.

The optimal ratio of laboratory staff to the expected number of procedures is debatable and naturally, economics is the obstacle. The ratio should be high, because embryologists can then spend enough time on quality, training and procedural details to ensure the standard of quality required for success.

The job description of embryologists should be clear and should exclude tasks that detract from their true work. The embryologist's job has two main and equally important parts. Firstly, performing gamete and embryo handling and culture procedures. Secondly, but equally important, maintaining full awareness of quality control standards, both, by performing routine checks and tests as well as by maintaining detailed logs of incidents, changes, unexpected changes, and counter measurements.

Across all these duties, the following jobs positions can be clearly defined: director, supervisor, senior embryologist, embryologist and trainee. their actual numbers varying according to the number of the annual procedures. There may be positions for others to do such as perform preimplantation genetic diagnosis (PGD), research and secretarial work. Some of these jobs do not exist in small centers.[7,10,11]

Infection Control

All the ART laboratories deal with microbiological risks, therefore, a stringent infection control protocol should be implemented. All the laboratory staff must be trained to reduce potential risks.

ART laboratories have the possibility of transmission of infectious disease to patients, staff and embryos. The highest possible levels of hygiene, cleanliness and waste management must exist, with a tidy laboratory, kept clean in accordance with the written standard operating procedures that state the cleaning of all equipment.

Ideally, work surfaces should be kept clear of paperwork and should be cleaned on daily basis, and as needed, during the routine work. Waste matter should be disposed of properly and should not stay overnight at the laboratory. Soiled laboratory and theater laundry should be dealt with quickly. Sharps disposal bins should be changed when two-thirds full or weekly, which ever happens first. Cupboard doors, drawers and walls should be cleaned twice a week. Laboratory floors should be cleaned daily with water or a mild detergent, such

JAYPEE BROTHERS

as 7X, if necessary, at the end of each shift and during work, if necessary.

Special attention should be paid to incubators, workstations, microscopes, refrigerators, heating blocks and warming ovens. There should be a dedicated place for paperwork.

At the beginning and end of a period of work, the work area should be tidied and cleaned thoroughly.[12,13]

Standard Operating Procedures

Standard operating procedure (SOP) is defined as detailed, written instructions to achieve uniformity of the performance of a specific function. There, the focus is set on repeatedly applying unchanged processes and procedures and its documenting.

The SOP should be written in a clear format that contains an introduction, the purpose of the SOP, details of the tasks described, date of writing, expected date of review, the name and designation of the writer, name and designation of the reviewer, and it should be signed by the authorized person. There should be a read and sign table attached to every SOP, where the names of all the involved staff should be in this table, and they should sign after reading the SOPs, which means that all the staff are aware of the SOPs content. The master copy should be in the laboratory and a soft copy should be available for quick revision and checking.

The SOPs should be available to all laboratory personnel, and should describe in details the procedures used, and the equipment and reagents required for each procedure. The SOP should include all the activities of the laboratory such as infection control, house-keeping, auditing and safety.[7,10]

Supplies

The choice of disposables and media in the ART laboratory is a very critical issue as the quality, storage and transporting of supplies will affect the pregnancy rate.

All supplies should be suitable for the intended purpose and the suppliers must comply to a contract with specific terms and conditions. All the disposables should be mouse embryo assayed, where possible, and CE or FDA marked. Each batch of media must have quality certification, must be mouse embryo assayed and a proven-track record. The delivery of media should be under controlled conditions and batch number must be recorded for quality assurance. In addition, all supplies should be tested with a suitable assay before use.[7,9,10,14]

Quality Control and Quality Assurance

The main objective of any ART laboratory is to achieve the highest pregnancy rate and highest live birth rate.[15] One of the most important factors contributing to this outcome is the creation of embryos from the patients' gametes. The viability of embryos created and transferred is a major determinant in achieving a successful pregnancy.

As the development and viability of embryos and gametes *in vitro* is compromised by contaminants and relatively small fluctuations in the environment, such as temperature and pH,[16,17] it is imperative that optimal culture conditions for gametes and embryos are attained and maintained in the ART laboratory. The purpose of implementing quality control (QC) and quality assurance (QA) programs is to ensure reproducibility in all techniques and performance in the ART laboratory,[18] and thus, to provide constant optimal conditions for gamete and embryo culture. Moreover, the diagnostic as well as the therapeutic role of the ART laboratory, can benefit from implementing QC and QA schemes.[19,20]

Quality control involves measures and activities undertaken to control the quality of the products, methods, equipment and environment to ensure that the laboratory is functioning properly, and is carried out to prevent undetected problems that could lead to a compromise in service to the patient. Internal quality control (ICQ) refers to the measures undertaken onsite.

Quality assurance is a comprehensive program that includes all activities and programs intended to ensure or improve the performance of the laboratory and thus, the quality of care to the patient. QA includes measures, such as record keeping, evaluation and education of the laboratory staff, result reporting, treatment auditing, incident reporting as well as QC methodology.

Both QC and IQC are systems that look within a particular testing site. In comparison, external quality assessment (EQA) looks at differences between different sites, testing the same analyte to ensure continuity of patient care between institutes and/or to ensure that these standards meet those of the accredited/regulatory body.

When setting a new ART laboratory, there are usually a large number of new and untested variables, which should be validated before use in clinical practice. This may apply to equipment, methodology, environment, consumables and culture media. The use of QA and QC to test these new variables will ensure that optimal standards of practice are attained from the outset of the new ART laboratory, thus ensuring that patient treatment is not compromised. In a prospective analysis, it was demonstrated that the employment of QC schemes identified a problem with the equipment before the treatment commenced when setting-up new ART laboratories.[20] These problems have likely led to a compromise in the pregnancy rate, if they had gone unchecked. As such, QC can give confidence to the scientific staff and thus, the whole IVF team that the gamete and embryo development are not compromised by the new laboratory facilities and procedures. The use of EQA, where possible, is also important to ensure that the laboratory practice and personnel in the new ART laboratory are comparable to the acceptable standards of other laboratories or preferably to the accredited/regulatory bodies.[21-24]

JAYPEE BROTHERS

Failure Mode and Effect Analysis

A failure mode and effects analysis (FMEA) is an inductive failure analysis used in industrial and service sectors, including ART laboratories for analysis of potential failure modes within a system for classification by the severity and likelihood of the failures. A successful FMEA activity helps a team to identify potential failure modes based on past experience and on common sense and logic, enabling the team to design those failures out of the system with the minimum effort and resource expenditure, thereby reducing development time and costs. Because it forces a review of functions and functional requirements, it also serves as a form of design review to erase weakness out of the design. It is widely used in the medical service. Failure modes are any errors or defects in a process, design, or item, especially those that affect the intended function of the product, service or process, and can be potential or actual. Effects analysis refers to studying the consequences of those failures on different system levels.[12]

Malpractice Insurance

Financial and emotional stresses often oppress patients seeking infertility treatment, and especially after failed cycles. This reflects into increasing number of complaints and legal cases. All of the medical, paramedical and scientific staff and the institution owning the laboratory should obtain an insurance policy of sufficiently high level and quality before the first day of operations. Litigation-prone issues need special consideration and include cancellation of a cycle, failure to achieve pregnancy, patient identification errors, interrupted cryostorage events, operation complications, and unexpected events.[4]

CONCLUSION

This chapter provides a general frame for setting-up an ART laboratory. The conditions of each laboratory should be studied separately. In general, establishing a successful ART laboratory is a complicated job that requires detailed design and cooperation among people of different fields in order to achieve a healthy environment for the patients, gametes and staff.

REFERENCES

1. Justis T, Kreigsmann B. The feasibility study as a tool for venture analysis. Business Journal of Small Business Management. 1979;17:135-42.
2. Georgakellos A, Marcis M. Application of the semantic learning approach in the feasibility studies preparation training process. Information Systems Management. 2009;26:3 231-40.
3. Young M. Feasibility studies. Appraisal Journal. 1970;38(3): 376-83.
4. Cohen J, Gilligan A, Garrisi G. Setting an ART laboratory. In: Gardner D, Howls C, Weissman A, Shoham Z (Eds). Textbook of Assisted Reproduction Technique Laboratory and Clinical practice. Matin Duntiz Ltd; 2001.pp.17-25.
5. Go K. Quality Control: A Framework for the ART Laboratory. In: Keel B, May J, Jonge C (Eds). Handbook of the Assisted Reproduction Laboratory. CRC press LLC; 2000.pp.253-69.
6. Commission Directive, laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use. 2003/94/EC.
7. Magli M, Van den Abbeel E, Lundin K, Royere D, Van der Elst J, Gianaroli L. Revised guidelines for good practice in IVF laboratories. Hum Reprod. 2008;23(6):1253-62.
8. Boone W, Johnson J, Locke A, Crane M, Price T. Control of air quality in an assisted reproductive technology laboratory. Fertil Steril. 1999;70(1):150-4.
9. The Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology. Revised Guidelines for Human Embryology and Andrology Laboratories. Fertil Steril. 2008;90:S45-59.
10. Huggs J. IVF: Laboratory management. In: Huggs J. IVF and Embryo Culture. University of Leeds; 2008.pp.20-47.
11. Mortimer D, Mortimer S. Human resources finding (and keeping) the right staff. In: Mortimer D, Mortimer (Eds). Quality and Risk Management in the IVF Laboratory. Cambrige University Press; 2005.pp.201-9.
12. Elder K, Baker D, Ribes J. Infection and contamination control in the ART laboratory. In: Elder K, Baker D, Ribes J (Eds). Infections, Infertility and Assisted Reproduction. Cambridge University Press; 2005.pp.303-31.
13. Junk S. Infection control in the IVF laboratory. In: Fleming S, Cooke S (Eds). Textbook of Assisted Reproduction for Scientists in Reproductive Technology. Viva Publishing; 2009. pp. 123-31.
14. Nijs M, Franssen K, Cox A, Wissmann D, Ruis H, Ombelet W. Reprotoxicity of intrauterine insemination and in vitro fertilization-embryo transfer disposables and products: a 4-year survey. Fertil Steril. 2009;92:527-35.
15. Alper MM, Brinsden P, Fischer R, Wikland M. Is your IVF programme good? Hum Reprod. 2002;17:8-10.
16. Hall J, Schemmil T, Cecchi M, Cohen J. The origin, effects and control of air pollution in laboratories used for human embryo culture. Hum Reprod. 1998;13:146-51.
17. Gardner DK, Lane M, Embryo culture systems. In: Trounson AO, Gardner DK (Eds). Handbook of in Vitro Fertilization. Boca Raton: CRC Press; 1999.pp205-64.
18. Wikland M, Sjoblom C. The application of quality systems in ART programmes. Mol Cell Endocrinol. 2000;166:3-7.
19. Matson PL. Internal quality control and external quality assurance in the IVF laboratory. Hum Reprod. 1998;13(suppl 4):156-65.
20. Cutting R, Pritchard J, Clarke H, Marrtin K. Establishing quality control in the IVF laboratory. Hum Fert. 2004;7:119-25.
21. Kastrop P. Quality management in the ART laboratory. Reprod Bomed Online. 2003;7:6 691-4.
22. Gilling-Smith C, Emiliani S, Almeida P, Liesnard C, Englert Y. Laboratory safety during assisted reproduction in patients with blood-borne viruses. Hum Rep. 2005;20:1433-8.
23. Carson B, Alper M, Keck C. ISO 9001:2000 Clause 4-Quality management system. In: Carson B, Alper M, Keck C (Eds). Quality Management Systems for Assisted Reproductive Technology. Taylor and Francis; 2004. pp. 20-24.
24. Mortimer D, Mortimer. Quality and risk management tools. In: Mortimer D, Mortimer (Eds). Quality and Risk Management in the IVF Laboratory. Cambrige University Press. 2005;118-34.

JAYPEE BROTHERS

for detailed information on obstetrics & gynecology books, visit our website **www.jaypeebrothers.com,** for detailed information on obstetrics & gynecology books, visit our

Available at all medical bookstores
or buy online at www.jaypeebrothers.com



JAYPEE BROTHERS
Medical Publishers (P) Ltd.
www.jaypeebrothers.com

Join us on facebook.com/JaypeeMedicalPublishers

Shelving Recommendation
OBSTETRICS & GYNECOLOGY

