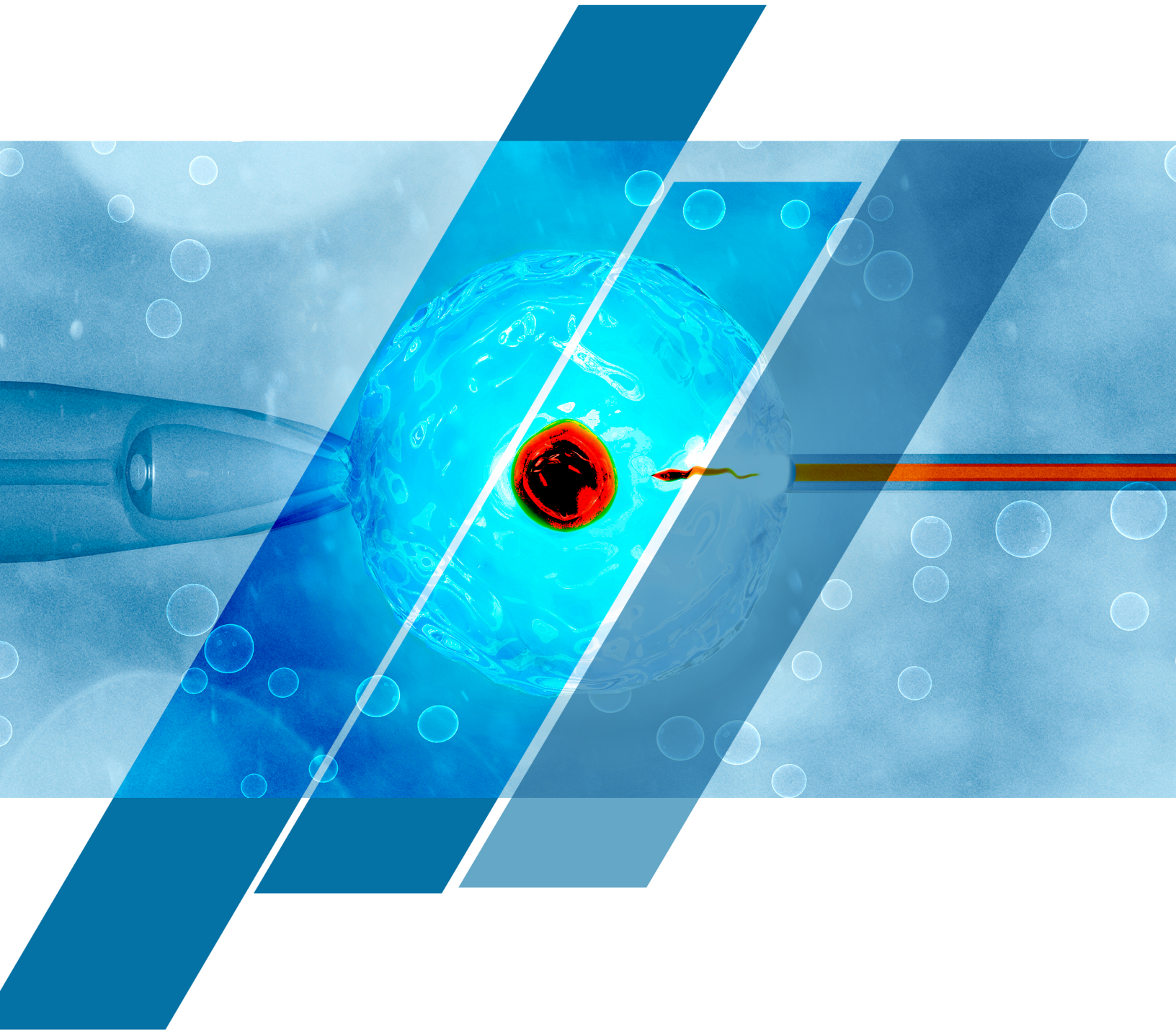


Advances in Assisted Reproduction Technologies



Editor:
Islam M. Saadeldin

Bentham Books

**Recent Advances in
Biotechnology**

(Volume 5)

***Advances in Assisted
Reproduction Technologies***

Edited by

Islam M. Saadeldin

*Research Institute of Veterinary Medicine
Chungnam National University
34134 Daejeon
Republic of Korea*

*College of Veterinary Medicine
Chungnam National University
34134 Daejeon
Republic of Korea*

&

*Department of Physiology
Faculty of Veterinary Medicine, Zagazig University
44519 Zagazig
Egypt*

Recent Advances in Biotechnology

Volume # 5

Advances in Assisted Reproduction Technologies

Editor: Islam M. Saadeldin

ISSN (Online): 2468-5372

ISSN (Print): 2468-5364

ISBN (Online): 978-981-5051-66-7

ISBN (Print): 978-981-5051-67-4

ISBN (Paperback): 978-981-5051-68-1

©2022, Bentham Books imprint.

Published by Bentham Science Publishers Pte. Ltd. Singapore. All Rights Reserved.

First published in 2022.

BENTHAM SCIENCE PUBLISHERS LTD.

End User License Agreement (for non-institutional, personal use)

This is an agreement between you and Bentham Science Publishers Ltd. Please read this License Agreement carefully before using the ebook/echapter/ejournal (“**Work**”). Your use of the Work constitutes your agreement to the terms and conditions set forth in this License Agreement. If you do not agree to these terms and conditions then you should not use the Work.

Bentham Science Publishers agrees to grant you a non-exclusive, non-transferable limited license to use the Work subject to and in accordance with the following terms and conditions. This License Agreement is for non-library, personal use only. For a library / institutional / multi user license in respect of the Work, please contact: permission@benthamscience.net.

Usage Rules:

1. All rights reserved: The Work is the subject of copyright and Bentham Science Publishers either owns the Work (and the copyright in it) or is licensed to distribute the Work. You shall not copy, reproduce, modify, remove, delete, augment, add to, publish, transmit, sell, resell, create derivative works from, or in any way exploit the Work or make the Work available for others to do any of the same, in any form or by any means, in whole or in part, in each case without the prior written permission of Bentham Science Publishers, unless stated otherwise in this License Agreement.
2. You may download a copy of the Work on one occasion to one personal computer (including tablet, laptop, desktop, or other such devices). You may make one back-up copy of the Work to avoid losing it.
3. The unauthorised use or distribution of copyrighted or other proprietary content is illegal and could subject you to liability for substantial money damages. You will be liable for any damage resulting from your misuse of the Work or any violation of this License Agreement, including any infringement by you of copyrights or proprietary rights.

Disclaimer:

Bentham Science Publishers does not guarantee that the information in the Work is error-free, or warrant that it will meet your requirements or that access to the Work will be uninterrupted or error-free. The Work is provided "as is" without warranty of any kind, either express or implied or statutory, including, without limitation, implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the results and performance of the Work is assumed by you. No responsibility is assumed by Bentham Science Publishers, its staff, editors and/or authors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products instruction, advertisements or ideas contained in the Work.

Limitation of Liability:

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

General:

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of Singapore. Each party agrees that the courts of the state of Singapore shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.

80 Robinson Road #02-00

Singapore 068898

Singapore

Email: subscriptions@benthamscience.net



CONTENTS

PREFACE	i
LIST OF CONTRIBUTORS	ii
CHAPTER 1 PHYSIOLOGY OF THE REPRODUCTIVE SYSTEM	1
<i>Mohamed M. Z. Hamada and Islam M. Saadeldin</i>	
GONADAL DEVELOPMENT AND SEX DETERMINATION	1
Gonadal Development	1
<i>In a Male Fetus</i>	2
<i>In a Female Fetus</i>	3
Sex Determination	4
<i>Primary Sex Determination</i>	4
MALE REPRODUCTIVE SYSTEM	7
Introduction	7
The Male Gonad (Testes)	8
Seminiferous Tubules	8
<i>Spermatogenic Process</i>	9
<i>Sertoli Cells</i>	12
Leydig Cells	13
<i>Androgen</i>	14
<i>Functions</i>	14
CONTROL OF TESTIS FUNCTIONS	15
Neuroendocrine Control	15
Pituitary Gonadotropins	17
<i>Effects of FSH on Sertoli Cells</i>	17
<i>Effect of LH on Leydig Cells</i>	17
Androgen	17
Inhibin and Activin	17
Testicular Temperature	18
<i>Mechanism Of Testicular Temperature Regulation</i>	18
<i>Cryptorchidism</i>	19
DUCT SYSTEM	19
Intra-testicular Ducts	20
Extra-testicular Ducts	20
Epididymis	20
Ductus Deferens (Vasa Deferentia)	21
Accessory Genital Glands	21
Seminal Vesicle	22
Prostate Gland	22
Bulbourethral Gland (Cowper's Gland)	23
PHYSIOLOGY OF MALE SEXUAL ACT	23
Parasympathetic Activity	24
Sympathetic Activity	24
Somatic Activity	25
FEMALE REPRODUCTION	25
Ovarian Cycle	25
<i>Follicular Phase</i>	25
<i>Ovulation</i>	33
<i>Luteal Phase</i>	35
Reproductive Cycle	37

(Estrus Cycle and Menstrual Cycle)	37
<i>Estrus Cycle</i>	38
<i>Menstrual Cycle</i>	39
Control of Reproductive Cycle	40
The Estrogen Action is Characterized by:	40
Progesterone also Regulates FSH and LH Secretion Through	41
<i>Hormonal Control of Ovarian and Reproductive Cycle</i>	42
Pregnancy	48
<i>Structure of the Ovulated Oocyte</i>	48
<i>Fertilization</i>	49
<i>Maternal Recognition of Pregnancy</i>	51
<i>Embryonic Development and Differentiation</i>	51
<i>Implantation</i>	52
<i>Placentation</i>	53
<i>Chorionic Gonadotropins</i>	54
<i>Placental Lactogen (PL)</i>	55
<i>Steroid Hormones</i>	55
<i>Relaxin</i>	56
<i>Hormonal Control of Pregnancy</i>	57
<i>Other Hormones</i>	57
CONCLUSION	58
CONSENT FOR PUBLICATION	58
CONFLICT OF INTEREST	58
ACKNOWLEDGEMENT	58
REFERENCES	58
CHAPTER 2 SPERM ASSESSMENT AND PROCESSING	60
<i>Krishnavignesh Lakshmanan, Deepa Onnarakatt, V. M. Thomas, Smisha Sridev,</i> <i>Anuja Gopalakrishnan and Poongothai Muthuswamy</i>	
INTRODUCTION	60
WHAT IS SPERM?	61
WHAT IS THE DIFFERENCE BETWEEN SPERM AND SEMEN?	62
Semen Collection	62
Semen Analysis	63
<i>Macroscopic Investigation</i>	65
<i>Microscopic Investigation</i>	67
<i>Sperm Morphology</i>	68
<i>Biochemical Evaluation</i>	72
<i>Why Semen Analysis has to be Repeated?</i>	72
<i>Sperm Counting Chambers</i>	73
<i>Semen Analysis by CASA Method</i>	74
<i>SQA</i>	74
Semen Processing	74
<i>Simple Wash Method</i>	75
Flowchart 2: Simple Wash Method	75
<i>Sperm Migration Based Methods</i>	76
<i>The Method Based on the Specific Density</i>	77
CONCLUSION	85
CONSENT FOR PUBLICATION	86
CONFLICT OF INTEREST	86
ACKNOWLEDGEMENT	86

REFERENCES	86
CHAPTER 3 SPERM FREEZING	90
<i>Mohammad A. Ibrahim</i>	
INTRODUCTION	90
EFFECT OF CRYOPRESERVATION ON SPERM CELLS	91
Bull Sperm Cryopreservation	93
<i>Bull Sperm Freezing using Protein-free Extenders</i>	94
<i>Bull Sperm Cryopreservation using Nanoparticles and Nanovesicles</i>	94
<i>Bull Sperm Lyophilization</i>	94
<i>Bull Sperm Encapsulation</i>	95
<i>Bull Sperm multi-thermal Gradient Freezing</i>	95
<i>Prediction of Bovine Sperm Freezability</i>	95
<i>Bovine Sperm Selection before Cryopreservation</i>	96
Buck (Goat) Semen Cryopreservation	96
Ram Semen Cryopreservation	97
Stallion Semen Cryopreservation	98
Cryopreservation of Camel-bull Semen	99
New Technologies to be Adopted for Animal Sperm Cryopreservation	100
<i>Freezing Small Numbers of Sperm</i>	100
<i>Solid Surface Vitrification (SSV)</i>	101
<i>Sperm Separation via Electrophoresis</i>	101
CONCLUSION	101
CONSENT FOR PUBLICATION	102
CONFLICT OF INTEREST	102
ACKNOWLEDGEMENT	102
REFERENCES	102
CHAPTER 4 CONTROLLED OVARIAN STIMULATION	112
<i>Firas Al-Rshoud and Tamara Darwish</i>	
THE OVARY	112
Embryology and Ovarian Reserve	113
Ovarian Physiology	113
FOLLICULAR STRUCTURE AND FUNCTION	113
The Process of Folliculogenesis, Ovulation, and Luteogenesis	114
<i>Primordial</i>	114
<i>The Primary Phase</i>	114
<i>The Secondary Follicle</i>	114
OVARIAN STIMULATION IN GENERAL	115
WHO Classification	115
Indications for Ovarian Stimulation	115
Anovulatory Infertility	116
Hypogonadotrophic - Hypogonadism	116
Polycystic Ovary Syndrome (PCOS) and Premature Ovarian Insufficiency	117
PCOS	117
Insulin Sensitizing Agents	118
Antiestrogens (Clomiphene Citrate and Letrozole)	118
Gonadotrophin Therapy	118
Laparoscopic Ovarian Drilling	119
POI	119
Diagnosis and Investigations	120
Subfertility and POI	120

Ovarian Stimulation (COS) in Vitro-Fertilization (IVF) Treatment	121
<i>In Vitro-Fertilization (IVF) Treatment</i>	121
Complications of Ovarian Stimulation: OHSS, M.P	121
CONCLUSION	122
CONSENT FOR PUBLICATION	123
CONFLICT OF INTEREST	123
ACKNOWLEDGEMENT	123
REFERENCES	123
CHAPTER 5 RECENT APPROACHES IN INTRAUTERINE INSEMINATION IN LIVESTOCK	125
Ömer Uçar	
INTRODUCTION	125
DESCRIPTION OF AI	126
Species Concerned	127
Personnel Involved	128
STRUCTURE OF SPERM AND A SUMMARY OF SEMEN HANDLING (FREEZE-THAWING)	128
Sperm Structure	128
Semen Freeze-thawing	128
INTRAUTERINE AI TECHNOLOGIES USED	130
Brief History of Reproductive Technologies	130
Main Intrauterine Insemination Techniques	131
AI PRACTICES IN DIFFERENT SPECIES	132
AI in Cows	132
<i>The Importance of Rectal Palpation (Experience And Timing)</i>	133
<i>The Importance of Pregnancy Detection (Experience, Method, and Timing)</i>	133
<i>The Importance of Oestrus Detection (Experience, Method, and Timing)</i>	134
<i>The Importance of Semen (Handling, Storage, Thawing, and Breed Chosen)</i>	135
<i>The Importance of Insemination (Experience, Site, Number, and Timing)</i>	135
<i>The Importance of Feeding (Storage, Silage, Supplements, Watering, Undernutrition, BCS)</i>	136
<i>The Importance of Management (Manure Disposal, Air Conditioning, Welfare, Culture, Exercise, Scratching)</i>	136
<i>The Importance of Health Services (Routine Controls, Vaccination, Culling)</i>	137
<i>The Importance of Reproductive Health (General Health, Libido, Semen, Breeding Soundness, Conception, Pregnancy, Birth, Abortus, Repeat Breeding, Inbreeding)</i>	138
<i>The Importance of Education and Organization (Continuous Training, Certification, Farmer/Breeder Associations, Feedbacks, Financial Supports)</i>	139
AI in Horses	139
AI in Buffaloes	139
AI in Camels	140
AI in Ewes	140
AI in Dogs	140
CONCLUSION AND FUTURE RECOMMENDATIONS	140
CONSENT FOR PUBLICATION	141
CONFLICT OF INTEREST	141
ACKNOWLEDGEMENT	141
REFERENCES	141
CHAPTER 6 ARTIFICIAL OOCYTE ACTIVATION	143
Thomas Ebner	

INTRODUCTION	143
PHYSIOLOGICAL AND ARTIFICIAL OOCYTE ACTIVATION	144
ARTIFICIAL OOCYTE ACTIVATION	145
Methods of AOA	146
Indications for AOA	148
Safety of AOA	149
CONCLUSION AND FUTURE PERSPECTIVES	149
CONSENT FOR PUBLICATION	150
CONFLICT OF INTEREST	150
ACKNOWLEDGEMENT	150
REFERENCES	150
CHAPTER 7 ADVANCES IN THE HUMAN PREIMPLANTATION EMBRYONIC CULTURE SYSTEM	153
<i>Manar M. Hozyen and Islam M. Saadeldin</i>	
THE EMBRYONIC CULTURE SYSTEM: A CHEMICAL PERSPECTIVE	153
Culture Media Formulation	154
Back to Nature and Let the Embryo Choose Theories	154
Oxygen	155
Macromolecules and Growth Factors Supplementation	156
THE EMBRYONIC CULTURE SYSTEM: A PHYSICAL PERSPECTIVE	158
Temperature	158
pH and Osmolality	158
Oil Overlay	161
Light	162
Single Embryo Culture and Groups Culture	163
Plasticware and Culture Dishes	164
Incubation Platforms	165
Static and Dynamic Nature	166
Deficiencies in the Current Culture Systems	168
Further Investigations	168
CONCLUSION	168
CONSENT FOR PUBLICATION	168
CONFLICT OF INTEREST	168
ACKNOWLEDGEMENT	169
REFERENCES	169
CHAPTER 8 ASSISTED HATCHING	174
<i>Jayesh Parasharam Shinde</i>	
INTRODUCTION	175
ASSISTED HATCHING AND ART	176
ASSISTED HATCHING	177
Methods for Assisted Hatching	178
<i>Mechanical Zona Pellucida Assisted Hatching</i>	178
<i>Zona Digestion using Enzyme Pronase (EZD)</i>	179
<i>Zona Drilling by Use of Acid Tyrode's Solution (ZD)</i>	180
<i>Laser-assisted Hatching (LAH)</i>	182
Applications of AH	184
<i>To Improve the Implantation</i>	184
<i>Laser-Assisted ICSI</i>	185
<i>Removal of Fragments and Necrotic Blastomeres</i>	185
<i>Cryopreservation</i>	186

<i>Hemizoma Assay</i>	186
<i>Preimplantation Genetic Testing (PGT)</i>	187
Assisted Hatching and Clinical Outcomes	188
Assisted Hatching and Monozygotic (MZ) Twinning	189
CONCLUSION	190
CONSENT FOR PUBLICATION	191
CONFLICT OF INTEREST	191
ACKNOWLEDGEMENT	191
REFERENCES	191
CHAPTER 9 OOCYTES AND EMBRYOS CRYOPRESERVATION	195
<i>Mohamed Fadel El Mohr and Islam M. Saadeldin</i>	
HISTORY OF OOCYTE AND EMBRYOS CRYOPRESERVATION	195
The Mechanism of Cryopreservation	199
<i>Slow Freezing</i>	199
<i>Rapid Freezing (Vitrification)</i>	201
Oocyte Cryo-preservation	204
Embryos Cryo-preservation	206
CONCLUSION	209
CONSENT FOR PUBLICATION	210
CONFLICT OF INTEREST	210
ACKNOWLEDGEMENT	210
REFERENCES	210
CHAPTER 10 REPRODUCTIVE CLONING	218
<i>Eman A. Hussien, Sara A. Mekkawy, Elham K. Eltahawy, Islam M. Saadeldin and</i>	
<i>Mohamed M. Omran</i>	
INTRODUCTION	218
HUMAN CLONING VS. ANIMAL CLONING	220
REPRODUCTIVE CLONING	223
Is Reproductive Cloning the Same as Stem Cell Research?	224
REPRODUCTIVE CLONING METHODS	227
Embryo Splitting	227
Tetraploid Complementation	227
Traditional and Alternative Methods of Cloning	227
Somatic Cell Nuclear Transfer (SCNT)	228
<i>The Source of the Donor Cell</i>	229
Induced Pluripotent Cells (iPS). Generation of Embryonic Stem Cell-like Cells	229
ADVANTAGES AND DISADVANTAGES OF REPRODUCTIVE CLONING	230
Advantages of Reproductive Cloning [42, 134, 135]	230
<i>Satisfy the Desire to have Children</i>	230
<i>To Treat Life-threatening Diseases</i>	230
<i>Genetic Modification</i>	230
<i>Speed up the Recovery Process</i>	231
<i>Compensation for Lost or Defected Organs</i>	231
<i>Contribution to Genetic Research</i>	231
Disadvantages of Reproductive Cloning [14, 133]	231
<i>Medical Malpractice Appearance</i>	231
<i>Lack of Diversity</i>	231
<i>A Faster Aging Process</i>	231
<i>Endangering People's Life</i>	231
<i>Interference with Nature</i>	232

<i>Making Division Between People</i>	232
<i>Problems with no Solutions</i>	232
<i>Abuse Toward Reproductive Cloning</i>	232
<i>Fears on Many Levels</i>	232
LEGALITY AND ILLEGALITY OF REPRODUCTIVE CLONING	233
Reproductive Cloning: Legal or Illegal	233
CONCLUSION	241
CONSENT FOR PUBLICATION	241
CONFLICT OF INTEREST	242
ACKNOWLEDGEMENT	242
REFERENCES	242
SUBJECT INDEX	274

PREFACE

More than 4 decades have passed since the birth of the first in vitro fertilized baby in 1978. The use of assisted reproductive technology (ART) to overcome infertility has increased exponentially with the simultaneous increase in the number of fertility centers in every part of the world. The use of ART continues to increase around the world, due to ever-increasing global access to infertility clinics in the treatment of different forms of infertility (like tubal disease, ovarian aging, or ovarian dysfunction).

This book captures the current and recent advances in assisted reproduction technology in humans and livestock in an easy and comprehensive way for the non-expert and the junior embryologists through simplifying each ART tool by providing definition and explanation, and how the methods are practiced and how to overcome troubleshooting, and showing the ARTs uses and significance.

This book will be an ideal reference for junior embryologists as it provides take-home messages for the current and recent ARTs. It contains ten chapters with a detailed explanation of normal reproductive physiology, male ARTs such as sperm retrieval and freezing, female ARTs such as oocyte activation, and cryopreservation, and finally embryo ARTs that include assisted hatching and cloning techniques.

Islam M. Saadeldin
Research Institute of Veterinary Medicine
Chungnam National University
Daejeon
Republic of Korea

List of Contributors

Anuja Gopalakrishnan	Department of Reproductive Medicine, Chennai Fertility Center and Research Institute, Chennai, Tamil Nadu, India
Deepa Onnarakatt	Department of Reproductive Medicine, Chennai Fertility Center and Research Institute, Chennai, Tamil Nadu, India
Elham K. Eltahawy	Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt
Eman A. Hussien	Biotechnology Program, Faculty of Science, Helwan University, Cairo, Egypt
Firas Al-Rshoud	Faculty of Medicine, Hashemite University, Zarqa, Jordan
Islam M. Saadeldin	Research Institute of Veterinary Medicine, Chungnam National University, 34134 Daejeon, Republic of Korea College of Veterinary Medicine, Chungnam National University, 34134 Daejeon, Republic of Korea Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt
Jayesh Parasharam Shinde	ART Fertility Clinic PVT LTD, B 404 Aishwaryam Comfort, Akrudi, Pune-411019, Maharashtra, India
Krishnavignesh Lakshmanan	Department of Reproductive Medicine, Chennai Fertility Center and Research Institute, Chennai, Tamil Nadu, India
Manar M. Hozyen	Ganin Fertility Center, Cairo, Egypt
Mohammad Ibrahim	Department of Theriogenology, Faculty of Veterinary Medicine, Alexandria Governorate 5424041, Egypt
Mohamed M. Z. Hamada	Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt
Mohamed Fadel El Mohr	Dr. Faris Medical Center for Infertility and Human Reproduction, Heliopolis, Cairo, Egypt Dar El Om for Infertility and Human Reproduction, Mokatam, Cairo, Egypt
Mohamed M. Omran	Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt
Ömer Uçar	Muğla Sıtkı Koçman University, Milas Veterinary Faculty, Division of Reproduction & A.I., Milas-Muğla, Turkey
Poongothai Muthuswamy	Department of Biotechnology, Dr. NGP Arts and Science College, Coimbatore, Tamil Nadu, India
Smisha Sridev	Department of Reproductive Medicine, Chennai Fertility Center and Research Institute, Chennai, Tamil Nadu, India
Sara A. Mekkawy	Biotechnology Program, Faculty of Science, Helwan University, Cairo, Egypt
Tamara Darwish	Faculty of Medicine, Hashemite University, Zarqa, Jordan

Thomas Ebner

Department of Gynecology, Obstetrics and Gynecological
Endocrinology, Johannes Kepler University, Altenberger Strasse 69, 4040
Linz, Austria

V. M. Thomas

Department of Reproductive Medicine, Chennai Fertility Center and
Research Institute, Chennai, Tamil Nadu, India

CHAPTER 1

Physiology of the Reproductive System

Mohamed M. Z. Hamada¹ and Islam M. Saadeldin^{1,2,3,*}

¹ Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt

² Research Institute of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

³ College of Veterinary Medicine, Chungnam National University; 34134 Daejeon, Republic of Korea

Abstract: The reproductive system of the living organism is the biological system made up of all the anatomical organs involved in sexual reproduction. This system involves the interaction of several fluids and hormones to regulate the functions of the reproductive system. The ultimate goal of the reproductive system is to successfully produce gametes (sperms and oocytes) to attain a combination of genetic material between two individuals, which allows for the possibility of greater genetic fitness of the offspring. In this chapter, we introduce the physiological process of gonadal development, male, and female reproductive system, embryo formation, and development to give the reader the basic concepts for application in the field of assisted reproductive techniques.

Keywords: Oocyte, Ovary, Physiology, Reproduction, Sperm, Testis.

GONADAL DEVELOPMENT AND SEX DETERMINATION

Gonadal Development

The gonads represent a unique embryological situation in that: the rudiments of all body organs except the gonads can normally differentiate into only one type of organ. For example, a lung rudiment can become only a lung, and a liver rudiment can develop only into a liver. On the other hand, the gonadal rudiment has two normal options. When it differentiates, it can develop into either an ovary or a testis. The path of differentiation taken by this rudiment determines the future sexual development of the organism. Before this decision is made, the mammalian gonad first develops through a bi-potential (indifferent) stage, during which time

* **Corresponding author Islam M. Saadeldin:** Research Institute of Veterinary Medicine, Chungnam National University, 34134 Daejeon, South Korea; Tel: 00821024817666; E-mails: islamms@cnu.ac.kr and islamaad82@gmail.com

it has neither female nor male characteristics (Figs. 1 and 2). The indifferent gonads consist of several components:

1. Coelomic epithelium, which is the precursor of Sertoli cells in males and granulosa cells in females.
2. Mesenchymal stromal cells, which are the precursor of Leydig cells in males and theca cells in females.
3. Germ cells that have migrated there from the yolk sac endoderm.

This assembly is organized into the indifferent gonads into two layers, cortex and medulla, and proceeds as follows:

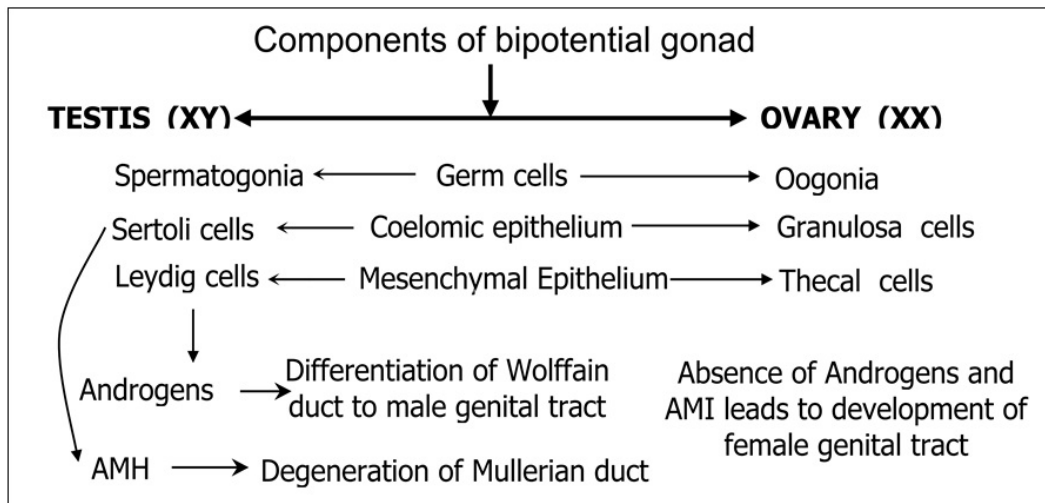


Fig. (1). Differentiation of the indifferent gonad components to their analog in both testes and ovary.

In a Male Fetus

Spermatogenic tubules begin to be formed at 6 weeks. This is followed by differentiation of the Sertoli cells at 7 weeks and Leydig cells at 8 to 9 weeks. At this point, the testes are structurally recognizable, and testosterone secretion begins.

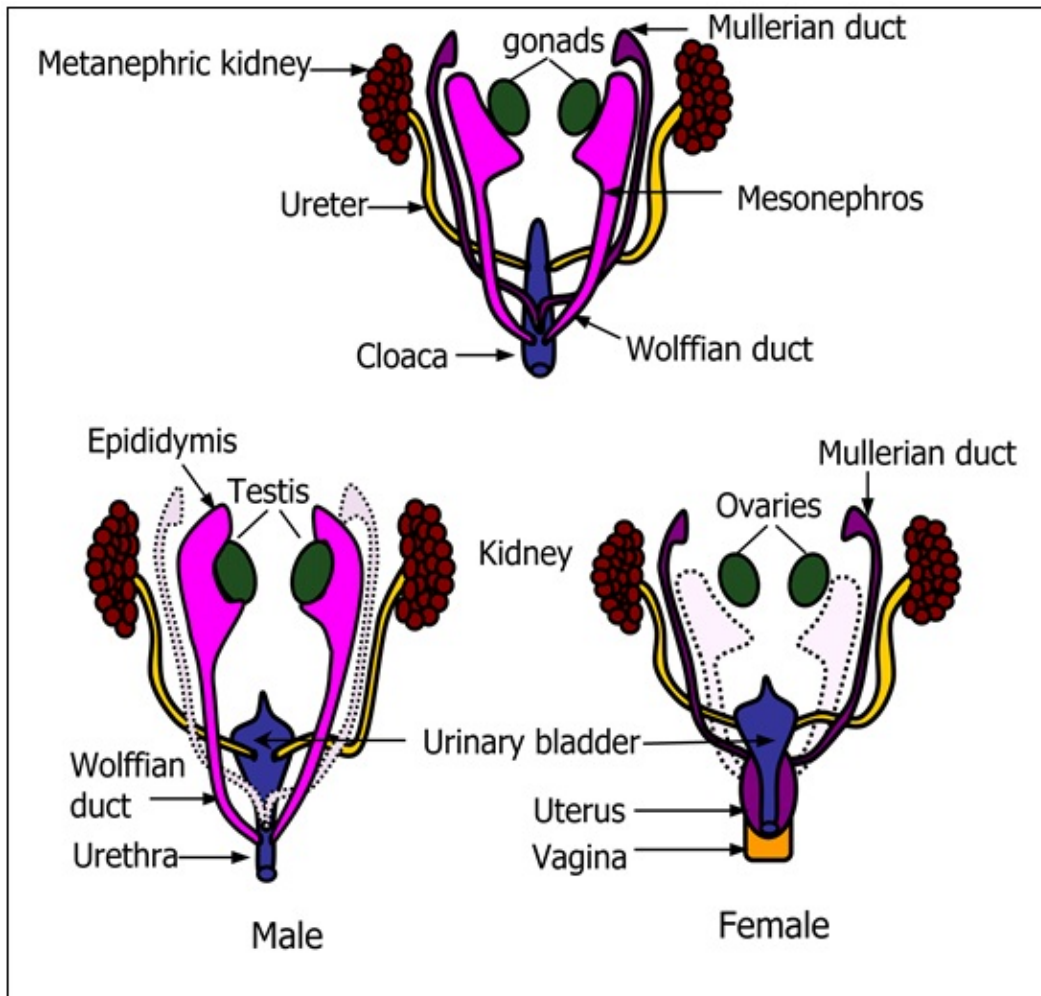


Fig. (2). Development of the gonads and their ducts in mammals. The upper figure represents the undifferentiated gonads and the presence of both male and female ducts. In the lower figures the male and female development is due to gonadal differentiation.

The germ cells become enclosed within the medulla, whereas the cortex is regressed. No known hormonal influences are required for the differentiation of the indifferent gonad into a testis till that stage. The urogenital groove (sinus) is the progenitor of the external genitalia. The Wolffian duct differentiates into the epididymis and the vas deferens.

In a Female Fetus

Differentiation of the indifferent gonad into an ovary does not start until 9 weeks

Sperm Assessment and Processing

Krishnavignesh Lakshmanan¹, Deepa Onnarakatt^{1,*}, V. M. Thomas¹, Smisha Sridev¹, Anuja Gopalakrishnan¹ and Poongothai Muthuswamy²

¹ Department of Reproductive Medicine, Chennai Fertility Center and Research Institute, Chennai, Tamil Nadu, India

² Department of Biotechnology, Dr. NGP Arts and Science College, Coimbatore, Tamil Nadu, India

Abstract: Semen analysis has become a definitive tool for understanding the male fertility factor. The volume of the ejaculation indicates the functioning and patency of testicular organs, whereas the quantity and quality of sperm define one's fertility profile. Thus, semen analysis is the cornerstone for diagnosis as well as for medical management. Semen comes with both cellular and non-cellular components. For the management of male infertility, it is necessary to have better quality sperm. As a result, semen processing has become an integral part of any medically assisted reproduction. There are different standardized preparation methods available that include sperm wash, swim-up technique, pelleting method, density gradient, *etc.* The aim of this chapter is to discuss semen analysis, its relevance in understanding male fertility factors, and the available sperm preparation methods. Based on the literature, it can be concluded that a proper semen assessment, according to the latest WHO guidelines, is mandatory for diagnosing male fertility issues. The appropriate sperm preparation method and good quality of sperms combined with the skill set of embryologists and environmental conditions will be the decisive factor in any IUI/IVF/ART success.

Keywords: Density gradient, Pelleting method, Semen analysis, Semen processing, Sperm wash, Swim-up technique.

INTRODUCTION

The creation begins with conception. Conception is achieved by two germ cells called sperms and oocytes. All conceptions are not achieved naturally, thus resulting in infertility. It turns out to be a global problem since it affects about 20% of couples worldwide. Male and female factors equally contribute to this condition. For the male fertility evaluation, a semen analysis (SA) is the primary screening procedure. It is advised to perform a minimum of two standardized

* **Corresponding author Deepa Onnarakatt:** Department of Embryology, Chennai Fertility Center and Research Institute, 79/129, Nelson Manickam Road, Aminjikarai, Chennai 600029, Tamil Nadu, India; Tel: +91 9840588473; E-mail: deeps688@gmail.com

semen analyses at an interval of 4 weeks apart. SA is repeated since semen parameters vary from time to time in one person as like other fluid parameters. It is also done to neglect technical or manual errors. World health organization (WHO, 2021) instructed to perform at least one diagnostic semen analysis before initiation of a treatment cycle. It has provided guidelines, reference values, and protocols to perform a semen analysis (Table 1) [1].

Table 1. WHO 2021 guidelines for seminal parameters and reference values [1].

Parameter	Lower reference value
Semen volume	1.4 ml
Total sperm number (10^6 per ejaculate)	39
Sperm concentration	16 M/mL
Total motility (PR + NP, %)	42%
Progressive motility (PR, %)	30%
Vitality (live spermatozoa, %)	54%
Sperm morphology (normal forms, %)	4%
pH	≥ 7.2
Peroxidase-positive leukocytes (10^6 per ml)	< 1.0
Immunobead test (motile spermatozoa with bound beads, %)	≥ 50
Seminal zinc (mol/ejaculate)	≥ 2.4
Seminal fructose (mol/ejaculate)	≥ 13
Seminal neutral glucosidase (mU/ejaculate)	≥ 20

Semen processing is a vital part of any IVF (*in vitro* Fertilization) treatment. It can be done for ongoing procedures as well as for cryopreservation for future use. It is directly related to the success of the procedure. A variety of semen preparation methods are followed in IVF laboratories. In this chapter, we are going to discuss various insights into sperm assessment and processing in detail.

WHAT IS SPERM?

Sperm is the fundamental unit of the male reproductive system. Sperm, illustrated in Fig. (1) is a haploid microscopic male reproductive cell. It is produced in the seminiferous tubules of testes, matures and stored in the epididymis, and transported through the ejaculatory duct along with the fluids secreted from the accessory glands [2].

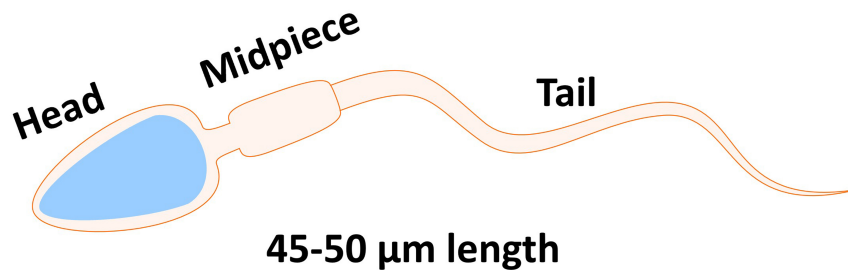


Fig. (1). Normal human sperm.

The head of mature sperm is made up of a well-defined nucleus and acrosome region. The nucleus contains the 23 chromosomes of parental DNA ranging from 30-35% while the acrosome contains proteolytic enzymes that constitute around 65-70%. Unlike most somatic cells, it lacks a large cytoplasm. The head is followed by a mid-piece that contains mitochondria, and a tail facilitates its mobility [3].

WHAT IS THE DIFFERENCE BETWEEN SPERM AND SEMEN?

Semen is a term that is often used interchangeably with sperm, but both are different, where semen refers to the seminal fluid that contains millions of sperm cells along with other plasma fluid. It can look white, yellow, or slightly greyish in color and viscous. The composition of the semen is of mature and immature sperm cells, white blood cells, and other non-cellular components like spermine, prostaglandins, electrolytes, *etc* [4].

Semen Collection

Semen collection is a vital step. Clear instructions should be given to the patients for collecting the entire sample in a wide-mouth non-toxic sterile container (Fig. 2). The patient can be asked to collect the sample through masturbation in a room near the andrology lab. If the patient faces difficulties in collecting samples, it may be advisable for taking phosphodiesterase (PDE) inhibitors (prescribed based on clinician's advice), home collection in a sterile container or a non-spermicidal condom, or through electroejaculation. After home collection, the sample should be transported to the examining laboratory within 45 minutes (avoid extreme temperature), since the semen analysis should be completed within 1-hour post ejaculation. The semen container can be kept at 37°C or room temperature (RT). The detailed reports should be maintained for every sample (Fig. 3). All semenology procedures should be carried out in an aseptic condition (Fig. 4) [1].

Sperm Freezing

Mohammad A. Ibrahim^{1,*}

¹ Department of Theriogenology, Faculty of Veterinary Medicine, Alexandria University, Alexandria Governorate 5424041, Egypt

Abstract: Sperm freezing is one of the reproductive technologies responsible for increasing domestic animals' fertility and preserving wild animal fertility over the last 100 years. This chapter will shed light on the current understanding of the effect of cryopreservation on sperm cells, advances in cryopreservation in terms of cryoprotectants and freezing protocols for bovines, caprines, ovines, equines, and camelids. Moreover, this chapter will shed light on other technologies needed to be adopted for different animal species.

Keywords: Cryopreservation, Cryoprotectants, Fertility, Sperm.

INTRODUCTION

Sperm freezing is essential for the distribution of superior genetics among domestic animal species and the preservation of wild animal species. Sperm freezing enables increasing sperm longevity and availability to inseminate animals in another part of the world or even in the future when the original donor is already dead. However, this at the expense that sperm cells thawed after freezing undergo alternation in their functionality, and intracellular and plasma membrane structures, which leads to compromised fertilizing ability.

Advances in sperm cryopreservation go around improving post-thawing sperm parameters to be as close to pre-freezing sperm parameters. In this context, a substantial amount of work has been dedicated to testing different cryoprotectants, additives, cooling rates, and freezing dose. It is worth noting that a freezing protocol that is suitable for one animal species does not essentially work for another. This is could be attributed to many reasons but primarily due to the different composition of sperm cell plasma membrane of different animal species. The cryotolerance of the sperm plasma membrane is determined according to membrane cholesterol to phospholipid ratios. In this context, species can be

* Corresponding author Mohammad A. Ibrahim: Theriogenology Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria Governorate 5424041, Egypt; Tel: 00201553330486; E-mail: mohammad.ibrahim@alexu.edu.eg

ordered, according to their plasma membrane cholesterol to phospholipid ratio, hence sperm cryotolerance is as following: human (0.99-0.83), rabbit (0.88), bull (0.40-0.45), ram (0.38), stallion (0.36), rooster (0.30), and boar (0.20-0.26) [1 - 5].

EFFECT OF CRYOPRESERVATION ON SPERM CELLS

As with any plasma membrane, the spermatozoa plasma membrane is built up of lipids and proteins. Lipids are in the form of phospholipids and cholesterol. The phospholipids are arranged as a dynamic bilayer. Each phospholipid is composed of a phospho-head group and two fatty acyl chains. The fatty acyl chains are varying in length and structure ranging from 14 to 22 carbons and contain 0–6 unsaturated bonds. Cholesterol is composed of four lipophilic carbon rings (associated with the fatty acyl chains) and a carbon side chain (associated with the head groups of the phospholipid). Therefore, cholesterol can fill in any gaps created in the membrane's core because fatty acyl chains have different carbon lengths or different degrees of unsaturation. Cholesterol, therefore, helps to stabilize the membrane at body temperature. When the spermatozoa undergo cooling, the lipid parts undergo a transition state called a phase transition, where they transform from being in a fluid state into a solid (gel) state. Cellular plasma membranes do not undergo the phase transition at a single temperature but over a range of temperatures. This is because the phase transition of each fatty acyl chain occurs at a different temperature. As lipids undergo the phase transition coalesces into 'icebergs', they leave their protein partners, which in turn coalesce to form protein clumps within the membrane, which significantly alters their functional capacities. As the temperature is reduced further, more and more lipid species undergo the phase transition and coalesce in these 'icebergs' of solid lipid, leaving a constantly smaller portion of the fluid membrane. Finally, upon reaching the lowest temperature of the membrane range of transition, the entire membrane is in the 'gel' state. At -5°C , the extracellular solute, the cells, and intracellular water are still not frozen but in a supercooled state. Between -5 to -15°C , the intracellular water is still in a supercooled state, but the extracellular medium starts to form ice crystals [6]. Due to concentration differences, the intracellular water will diffuse to the extracellular medium and freeze. If the cooling speed beyond -15°C is slow, almost all intracellular water will diffuse out to an external medium leading to dehydration and shrinkage of the cell, and ultimately a hyperosmotic shock. However, if the cooling speed is fast, ice crystal formation will start due to the presence of intracellular water that did not get out [7]. Therefore, sperm cooling and freezing rate should be fast sufficiently to circumvent spermatozoa dehydration and shrinkage but slow adequately to dodge intracellular ice crystals formation [7]. Moreover, cooling, and freezing temperatures lead to the compromise of sperm ATP-dependent ion channels of potassium, sodium, magnesium, and calcium leading to depolarization and an

increase in permeability of plasma membrane and mitochondria, leading to premature capacitation, cell death, peroxidation, loss of lipids from the plasma membrane, and release of reactive oxygen species (ROS) [8].

The objective of cryopreservation protocol is the survival of sperm cells during freezing and after thawing. During thawing, sperm cells undergo rehydration, osmotic stress, and ultimately plasma membrane disruption. Therefore, optimizing sperm cryopreservation consists of optimizing three main components: sperm cryoprotectant, cooling/freezing rate, and thawing rate.

Cryoprotectants are used to decrease cryoinjury during freezing and thawing. Cryoprotectants are classified according to their ability to penetrate the sperm cell into permeating or non-permeating cryoprotectants. *Permeating cryoprotectant* is a type of cryoprotectant able to penetrate the sperm cell membrane, consequently, they change the viscosity of the cytoplasm and decrease the concentration of intracellular electrolytes. Therefore, upon subjecting sperm cells to freezing temperatures, they will cause dehydration of the sperm cell and reduce the formation of intracellular ice and minimize the extent of osmotic shrinkage of sperm cells [9, 10]. Glycerol is generally the most used permeating cryoprotectant in mammalian spermatozoa cryopreservation. Also, ethylene glycol and dimethylacetamide have been used as penetrating cryoprotectants [11]. Glycerol and other permeating cryoprotectants can penetrate the sperm cells, therefore it is relatively toxic to the sperm cells. Since cytoplasm viscosity of sperm cell differs between animal species [12], therefore, glycerol concentrations tolerability depend on animal species. For example, bull and boar semen is relatively tolerate higher concentration of glycerol compared with stallions [13]. *Non-permeating cryoprotectants* are a type of cryoprotectants that do not penetrate the sperm cell membrane. It acts extracellularly by stabilizing the plasma membrane, decreasing the freezing point of the extracellular compartment, therefore, lessening formations of ice crystals around the sperm cells [14]. Non-permeating cryoprotectants include sugars such as lactose or trehalose or dextran, and proteins [15]. The protein of animal sources has been used as a cryoprotectant, as chicken egg yolk and fat-free skimmed milk are widely utilized for different animals' sperm cryopreservation. However, there is an increasing interest to use animal-free cryoprotectants to avoid risks for viral or other disease transmissions.

As previously mentioned, since sperm cell wall composition is different between species, hence does each animal species sperm prefer a particular freezing medium? what is the ideal concentration of the ingredients? The next sections will discuss the current understanding of cryopreservation protocols for different animal species.

Controlled Ovarian Stimulation

Firas Al-Rshoud^{1,*} and Tamara Darwish¹

¹ Department of Obstetrics and Gynecology, Faculty of Medicine, Hashemite University, P.O Box 330127, Zarqa 13133, Jordan

Abstract: For conception to occur, whether naturally or via assisted reproductive technology (ART), three things are required; a healthy sperm for fertilization, an ovum released from both ovaries (in the fallopian tubes for natural pregnancy or in the laboratory in IVF/ICSI), and a healthy place for the embryo to implant (a healthy receptive endometrial cavity), so any defect in any of the aforementioned pregnancy requirements will affect the achievement of pregnancy in both types; the natural one or that resulting from ART [1]. Ovarian stimulation is used to overcome any ovulatory disorder or in ART to collect as many eggs as possible (10-15 eggs at least) [2] to maximize the chances of pregnancy. Assessment of ovarian reserve is the first and most important step in ovarian stimulation, as this will indicate the proper method and dose needed for ovulation induction. We test for ovarian reserve using Biomarkers (FSH and AMH) and physical markers (antral follicles count(AFC)) by transvaginal ultrasound(TVS). Both AMH and AFC have similar accuracy in assessing ovarian reserve and are both superior to FSH as they have no cyclic variations [3]. This chapter will discuss all aspects of the physiology of the ovulation process and ovarian stimulation.

Keywords: Anovulation, ART, Ovarian stimulation.

THE OVARY

The human ovary is composed of the following; a) the hilum; b) the outer cortex; c) the central medulla. The hilum comprises the blood supply, nerves, and hilus cells. At the embryonic stage, the ovary is part of the mesovarium. The ovarian cortex consists of two parts, the outer layer (the tunica albuginea) and the inner one (ovarian follicles are implanted in stromal tissue). Further, mesonephric is the origin of the central medulla.

* Corresponding author Firas Al-Rshoud: Department of Obstetrics and Gynecology, Faculty of Medicine, Hashemite University, P.O Box 330127, Zarqa 13133, Jordan; Tel: 00962777682626; E-mail: reshoud75@yahoo.com

Embryology and Ovarian Reserve

It is well known that in average cases the primitive ectoderm is the origin site for human germ cells after they develop and migrate to the gonadal ridges (the migration takes place within 5 weeks of gestation). At this time window, the gonadal ridges and mesonephric ducts formulate. The process of migration entails the proliferation of germ cells followed by mitosis. Importantly, the variation of different gonads into either ovary or testis is controlled by genes-related sex chromosomes. However, at this early stage, rapid mitotic germ cell multiplication takes place in which 6-7 million oogonia develop (in the ovarian cortex), which is the highest number or the peak number, keeping in mind that this takes place by 20 weeks gestation. After delivery, the number of eggs decreases with age reaching puberty with less than one million and menopause with around 1000 eggs [4].

Ovarian Physiology

The cyclic activity in women is their reproductive capacity; a characteristic that mirrors the development of dominant follicles. In normal conditions, in each menstrual cycle, a single dominant follicle is produced by the ovaries, in which, this follicle plays a major role during the follicular phase; the production of estradiol. Afterward, ovulation takes place. The dominant follicle transforms into the corpus luteum, through which progesterone production takes place (luteal phase). Together, estradiol and progesterone are needed to facilitate the implantation of the human embryo. Hence, a thorough understanding of the life cycle of dominant follicles will facilitate an understanding of female fertility. This section will briefly discuss the following aspects: Follicular structure and function, the process of folliculogenesis, ovulation, and luteogenesis.

FOLLICULAR STRUCTURE AND FUNCTION

Folliculogenesis resembles the maturation of the ovarian follicle. This follicle is comprised of a shell composed of somatic cells consisting of the immature oocyte and represents the transformation of small primordial follicles into large preovulatory follicles and atresia of the remaining ones. This takes place monthly during the menstrual cycle. Folliculogenesis is divided into two stages; the first stage is gonadotropin-independent while the second stage is gonadotropin-dependent. The first stage represents the development and differentiation of the oocyte and it is organized by the production of growth factors through paracrine mechanisms. The second stage is follicular size increment until it reaches around 25-30 mm, and is controlled by FSH, LH, along with growth factors.

The Process of Folliculogenesis, Ovulation, and Luteogenesis

This process entails the maturation of the follicle from primordial through the secondary follicle.

Primordial

The primordial phase takes place at 18–22 weeks intrauterine, at which the ovarian cortex has its peak number of follicles (4 to 5 million in average case), however, the highest number of follicles could reach 6 to 7 million. These primordial follicles consist of immature oocytes separated from ovarian stroma by the basal lamina and supported by granulosa cells. These cells are dormant, and they can stay at the dormant stage for up to 50 years. Thus, it explains the fact that ovarian cycle length precludes this period. Hence, before birth, the follicles are reduced to around 180,000 by puberty. However, by quality, only 400 follicles reach the preovulatory phase, and by menopause, around 1,000 follicles remain. Thus, it is plausible that early and late menopause are bound by the number of populations at birth, however, no definite evidence is reported. Of note, when primordial cells 'wake up', the initial recruitment phase would take place mediated by hormones and growth factors.

The Primary Phase

In this phase, the activation of the ovarian follicle takes place. The granulosa cells that are around the primordial follicles change their morphological flat features to a cuboidal structure, signposting the launching of the primary follicle, and genes are then transcribed. Besides, paracrine mechanisms begin to initiate the signaling between the follicle and the oocyte. The oocyte and the follicle develop and grow to nearly 0.1 mm in diameter.

The primary follicle during this stage creates and develops follicle-related stimulating hormone (FSH) receptors, in addition, the oocyte will be surrounded by the zona pellucida (A glycoprotein polymer), to form a buffer zone between the oocytes and granulosa cells. The zona pellucida will be kept with the oocyte when the ovulation process is completed as it releases enzymes that enhance the ability of the sperm to penetrate the ovum.

The Secondary Follicle

At this stage, the oocyte-secreted signals will gather around the theca cells; the outermost layer of the follicle, the basal lamina, and undergo cytodifferentiation and will be surrounded by these cells to emerge as the theca externa and theca

CHAPTER 5**Recent Approaches in Intrauterine Insemination in Livestock****Ömer Uçar^{1,*}**¹ *Muğla Sıtkı Koçman University, Milas Veterinary Faculty, Division of Reproduction & A.I., Milas-Muğla, Turkey*

Abstract: In the present chapter, the recent studies on artificial insemination (AI) of livestock (large and small) animals via the intrauterine route are summarized. For this, intrauterine inseminations especially in cattle, horse, camel, buffalo, sheep, goat, dog, and cat species will be considered in detail. Brief data on inseminations in other species (pig, turkey, hen, honeybees, silkworm) is also given.

Once semen sample is collected from sexually mature and clinically healthy breeding male animals, it has to be kept alive (mainly by dilution and cooling) first and then used either immediately (fresh) or stored (chilled/frozen) until being used in oestrous females. During insertion of the semen (particularly stored ones), it is necessary to place the inseminate in the vicinity of the ovum (Graafian follicle) as close as possible. Hence, fertility results of insemination are always higher when semen is deposited intrauterine. However, this is not as easy as we would expect, due mainly to the anatomical structure of females (cat, dog, sheep, and hen) or viability of semen (fish and camel).

To overcome this female- or male-originated limiting factors of fertility, various novel approaches have been reported towards acceptable rates of fertility outcome. Hence, these recent insemination techniques are outlined herein.

Keywords: Animal, Artificial insemination, Breeding, Intrauterine, Livestock, Review.

INTRODUCTION

In a broad sense, reproduction aims to sustain the life of living creatures via bearing viable and productive offspring [1]. In animals, the duration of natural mating takes a very short time, only a couple of seconds (cattle, sheep, horse, hen) or so (camel, cat, turkey, fish). The exceptions are mating-lock ('tie-to-tie') in

* **Corresponding author Ömer Uçar:** Muğla Sıtkı Koçman University, Milas Veterinary Faculty, Department of Clinical Vet. Sci., Division of Reproduction & A.I., 48200-Milas, Muğla, Turkey; Tel.: 00 90 252 2115829; E-mail: omeurucar@mu.edu.tr

dogs (5-25 minutes long), a long mating period in camels (generally 7-15 minutes or occasionally day-long), or several matings (by 7-10 males) during ‘mating fly’ in honeybees (a couple of minutes) [2, 3]. Alternatively, however, the AI technologies are also practiced largely in livestock animals (mostly in cow, ewe, doe, sow, mare as well as in turkey, fish, honeybees, and silkworm). The most common practice of AI is seen in cattle (Figs. **1a**, **1B**) due mainly to achieving genetic improvement in calves (Figs. **1c**, **1d**) by spreading especially males’ desirable genetic characteristics (high milk yield and heavier calves born) and preventing mating-related diseases (Brucellosis, IBR/IPV, Tuberculosis, *etc.*) [4]. AI is one of the fastest ways to control diseases and increase individual (offspring) genetic characteristics on a national scale [5].



Fig. (1). AI caves in Brown Swiss (1a), Simmental (1b), or their crossed breeds (1c and 1d, resp.).

Conventional AI technologies include mostly the rectovaginal transcervical route in large animals (cattle, buffalo, camel), while the transcervical route is used both in large (horse, cattle) and small animals (dog). However, surgical methods of AI are also practiced mainly in small animals (dog, cat, sheep, goat).

Herein, a brief explanation of AI, relevant species, and people involved are given below.

DESCRIPTION OF AI

In short, AI may be described as the placement of an adequate dose (inseminate) of viable semen collected from the sire male animal to the relevant part of the genital tract of the oestrous female by using appropriate insemination techniques and methods [6, 7]. Globally, it is most commonly applied in cattle in numerous

countries (USA, UK, Denmark, Germany, Russia, Turkey, Israel, Japan, Australia, *etc.*) [2 - 4]. For guiding the routine conduct of AI practices in livestock animals [3], reproductive physiological values (oestrus, ovulation time, viabilities of sperm and ovum, optimal insemination time) of different species (cow, mare, ewe, doe, sow, bitch, queen, hen/turkey) are given in Table 1.

Table 1. Reproductive physiological values of various livestock species*.

Species	Physiological values in livestock				
	Oestrus	ovulation time	Viabilities of gametes		optimal insemination time
			Sperm	Ovum, h	
Cattle	12-18 h	10-12 h after oestrus ends	24-36 h	20-24	The second half of oestrus or final 1/3 period
Horse	4-7 day	1-2 days after oestrus begins	72-96 h	6-8	1 day before ovulation or near ovulation
Sheep	30-36 h	24-30 th h after oestrus begins	30-48 h	16-24	16-24 th h after oestrus begins
Goat	30-36 h	24-30 th h after oestrus begins	30-48 h	16-24	16-24 th h after oestrus begins
Pig	60-72 h	36-48 th h after oestrus begins	36-48 h	8-10	16-24 th h after oestrus begins
Dog	9 day	36-48 th h after oestrus begins	4-6 day	48-72	48 th and 96 th h after oestrus begins
Cat	6 day	48-72 nd h after mating	24-36 h	8-16	nearly 40 h after hCG administration
Poultry (a hen)	none	Every day at certain times	30 d in the female genital tract	1-2	Once a week (afternoon)

*Slightly modified from [3].

Species Concerned

AI can be performed mainly in ruminants (cattle, buffalo, sheep, and goats), pigs, and horses as well as other species such as bees and silkworms. Further, it is also performed in certain scale fish (rainbow trout) [1 - 3, 8, 9]. However, AI is prohibited especially in thoroughbred (British) horses and AI foals are not registered officially [1, 4]. An exception to this is the disease control for dourine (venereal trypanosomiasis) and infectious anemia by equine AI in some eastern European countries [4].

Artificial Oocyte Activation

Thomas Ebner^{1,*}

¹ Department of Gynecology, Obstetrics and Gynecological Endocrinology, Johannes Kepler University, Altenberger Strasse 69, 4040 Linz, Austria

Abstract: Against all expectations, the presence of a carefully selected normal spermatozoon does not guarantee oocyte activation/fertilization. In contrast, some ICSI cycles will have to face no or low fertilization in several consecutive cycles. Both sperm- and oocyte-derived problems may account for such a dilemma. In case of physiological activation problems, any artificial increase in ooplasmic calcium could rescue the fertilization process. Such approaches are summarized under the term artificial oocyte activation (AOA). AOA can be achieved by modified ICSI techniques, piezoelectrical manipulation, or chemical stimuli. Amongst these approaches, the latter is the currently most accepted one in IVF laboratories around the world and particularly the Ca²⁺-ionophores ionomycin and calcimycin are the most extensively studied agents. Recently, a ready-to-use ionophore (A23187) has been introduced which is CE-marked and as such will assist in the standardization of AOA techniques. There is growing evidence that for proper indications usage of AOA can be considered quite safe. This conclusion is based on studies on morphokinetics, chromosome segregation, and gene expression. More importantly, available neonatal and neurodevelopmental data are reassuring. However, since artificial oocyte activation rarely results in physiological Ca²⁺ oscillations and is not beneficial for all patients with a suspected activation deficiency these techniques should not be used without profound indication.

Keywords: Activation failure, Calcimycin, Fertilization failure, ICSI, Ionomycin, Ionophore.

INTRODUCTION

In a recent report, the ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine [1] stated that in a clinical laboratory setting it is highly recommended that Performance Indicators (PI) are evaluated. On the one hand, this would allow for the systematic analysis of the lab's contribution to patient care and, on the other, these PIs could represent an important instrument within an existing Quality Management System. More importantly, particular Key

* **Corresponding author Thomas Ebner:** Department of Gynecology, Obstetrics and Gynecological Endocrinology, Johannes Kepler University, Altenberger Strasse 69, 4040 Linz, Austria; Tel.: ++43 05768084 24600, Fax.: ++43 05768084 24604; E-mail: Thomas.Ebner@Kepleruniklinikum.at

Performance Indicators (KPI) such as ICSI degeneration rate, fertilization rate, or blastocyst development rate would prove useful to perform internal and external quality control. By ascertaining such KPIs embryologists could not only set minimum competency limits but also aspirational goals which would assist benchmarking with others.

According to these figures [1] normal (two polar bodies and two pronuclei) fertilization rate after conventional IVF should be between 60% (competency value) and 75% (benchmark value). Obviously, higher fertilization rates are expected for ICSI (65%-80%). These rational expectations are based on the mere fact that with the ICSI technique a single spermatozoon is directly injected into the ooplasm [2] which avoids some of the physiological barriers of the cumulus-oocyte-complex. ICSI, however, bears the risk that gametes are accidentally chosen for injection which shows deficits in its genetic constellation, phospholipase C zeta (PLC ζ) content, protamine ratio, the integrity of centrosome, and/or DNA methylation. Since such a scenario would have an impact on further outcomes, embryologists tend to select sperms considered for usage in the most physiological way [3]. Such selection processes aim for sperms with optimal morphology at higher magnification (IMSI), high sperm head birefringence, completed maturation (so-called pICSI), and/or DNA intactness.

However, even in the presence of a presumably functional spermatozoon that has been processed and identified by a “physiological” selection method oocyte activation and fertilization cannot be taken for granted. In fact, approximately 10% of all ICSI cycles show fertilization problems and 3% result in complete fertilization failure. As a consequence, some patients suffer from repeated fertilization failure which leads to cycle cancellation in the presence of normal sperm parameters and good ovarian response.

For affected couples, artificial oocyte activation (AOA) is the method of choice to restore fertilization and in fact, sometimes is the only way to achieve embryo transfer and eventually pregnancy. It should be kept in mind that AOA techniques should not be applied routinely since they are no physiological approaches at all.

PHYSIOLOGICAL AND ARTIFICIAL OOCYTE ACTIVATION

Before one could further focus on AOA and understand its mechanisms it is of importance to bring physiological oocyte activation to mind. In contrast to the natural conception or conventional IVF ICSI bypasses several physiological steps such as cumulus cell penetration, binding to the zona protein 3 receptor, active passage through the perivitelline space, and Izumo 1 to Juno binding once the membranes of the gametes fuse. A sperm entering the oocyte causes an elaborated

reaction in the same. In detail, time-lapse sequences of early preimplantation development revealed that the first sign of oocyte activation is the extrusion of the second polar body (on average 3.3h post-injection) followed by the formation and juxtapositioning of the pronuclei (6.3h and 8.5h, respectively) [4].

These events observed at light-microscopical resolution parallel physiological processes at the molecular level. Of note, the sperm-derived oocyte activation factor, an enzyme known as PLC ζ , enters the ooplasm upon fusion or injection. It then binds to membrane-bound phosphatidylinositol-biphosphate (PIP₂) which in turn cleaves into two molecules, diacylglycerol (DAG) and inositol-3-phosphate (IP₃). While DAG is involved in zona reaction to prevent polyspermy the latter compound is driving oocyte activation.

The produced IP₃ interacts with its receptors located at the membrane of the smooth endoplasmic reticulum (sER) whereupon the release of Ca²⁺ ions from inside the sER is initiated. This Ca²⁺ flux is modulated in a time-dependent manner so that it is literally presented in an oscillating mode. sER Ca²⁺-depletion is unlasting and in order to avoid complete loss of calcium the oocyte uses Ca²⁺ sensors that monitor available Ca²⁺ within the sER and in case a limited or even drastic depletion of the same is detected calcium entry from outside the egg is triggered to refill sER storages. In order to maintain the oscillatory activity of calcium over a longer period of time mechanisms have to exist which ensure restoration of the Ca²⁺- level to baseline after every single peak. This is mostly done *via* ion pumps, exchanger proteins, or calcium uptake into mitochondria (for review see [5]). Finally, oscillations cease once the two pronuclei are formed and they are noticed again immediately before every mitosis [6].

ARTIFICIAL OOCYTE ACTIVATION

Any deviation in the above-mentioned crucial biochemical processes and molecules involved, such as PLC ζ , PIP₂, or IP₃, would automatically cause a reduction or loss of intracellular Ca²⁺, in particular the absence of calcium oscillations. Such a scenario could also be the result of oocyte-related problems, *e.g.* in case the ooplasm is immature (which in turn would result in maldistribution of sER) or in case IP₃-receptors are not functional. However, irrespective of the fact whether cytosolic calcium deficiency is caused by sperm- or oocyte-derived problems it will have a profound impact on cell physiology, and as a consequence, the egg remains unfertilized. As long as calcium is the underlying cause of the dilemma any AOA technique would assist oocyte activation and fertilization by literally requisitioning extracellular Ca²⁺ from the culture medium to compensate for the observed loss. It has to be kept in mind that the vast majority of such artificial methodologies do not generate physiological

CHAPTER 7

Advances in the Human Preimplantation Embryonic Culture System

Manar M. Hozyen^{1,*} and Islam M. Saadeldin^{2,3,4,*}

¹ Ganin Fertility Center, Cairo, Egypt

² Research Institute of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea

³ College of Veterinary Medicine, Chungnam National University; 34134 Daejeon, Republic of Korea

⁴ Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt

Abstract: The embryonic culture system is the main part of *in-vitro* embryonic development. The culture system consists of chemical factors such as the chemical composition of the culture media and physical factors such as temperature, pH, oil overlay and, incubation systems. The optimal culture conditions are needed to support embryonic development *in-vitro*, increase pregnancy, implantation rates, and decrease the level of pregnancy loss. Any suboptimality in culture conditions may impair embryonic development and affect subsequent viability. In this chapter, we will focus on the current culture system in the field of assisted reproductive technology (ART), passing by current culture media formulations, deficiencies, culture devices used, and the areas that need further investigations.

Keywords: Culture dishes, Culture media, Culture system, Embryo culture, Oil overlay, pH.

THE EMBRYONIC CULTURE SYSTEM: A CHEMICAL PERSPECTIVE

Culture system is the surrounding environment of the gametes and embryos growing *in vitro* based basically on the normal composition of the female reproductive tract, in which embryos would normally be exposed [1, 2]. Mimicking nature could support preimplantation embryonic development *in vitro*, which might result in higher post-implantation outcomes (pregnancy, implantation, and live birth rates).

* Corresponding authors Manar Mohamed Hozyen and Islam M. Saadeldin: Ganin fertility center, Egypt; E-mail: manarnrc@gmail.com and Research Institute of Veterinary Medicine, Chungnam National University, 34134 Daejeon, South Korea; Tel: 00821024817666; E-mails: islamms@cnu.ac.kr and islamsaad82@gmail.com

Culture Media Formulation

Many commercial media are developed for assisted reproductive technologies (ART), to support embryonic development [1, 3, 4]. Basically, the chemical composition of *in-vitro* fertilization media is composed of:

1. Water, which is the major component, about 99% of the media [5].
2. Ions such as potassium, which has an important role in sperm capacitation, chloride ions, which affect mRNA or protein synthesis, and calcium as an essential ion for the compaction stage of the embryos [6].
3. Carbohydrates and other components such as amino acids, vitamins, nucleic acid precursors, chelators, antioxidants, antibiotics, buffer system, protein/macromolecules, hormones, and growth factors [1, 4, 7].

Each component of the media has a specific role to complete the orchestra. Different commercial culture media have different compositions or nutrient concentrations [3, 4]. The exact media formulations and their concentrations are usually not disclosed by the manufacturers due to commercial competition [3]. Up to date, the optimum medium formulation is still unknown and needs more investigations [3]. Hence, each IVF laboratory should choose and validate the media that match their needs.

Back to Nature and Let the Embryo Choose Theories

The development of the embryonic culture media passed with many evolutionary steps [6]. Starting with: The introduction of the culturing idea, *in vitro* first successful fertilization of human gametes in simple media by Robert Edwards, and further modifications to the current complex human culture media forms [6, 8]. Now, the design of culture media emerged from two distinct main theories. The first one is a “back to nature” or “sequential culture media”, which developed to mimic the oviduct and uterine component as the natural environment surrounding the embryos. This approach involves refreshing the embryos from one media, which supports pre-compaction embryo development to another that supports post-compaction development regarding the physiology and embryonic need [6, 9]. Although the beneficial use of the sequential media; raises the concern about the stress that evolved from moving the embryos from one media to another [9]. To eliminate this concern, the other approach has been emerged as “let the embryo choose” or “single-step media.” In this approach embryo, itself will choose its requirements from the surrounding media. As the media contains the concentration of nutrients and adequate substrates required for embryonic development from fertilization to the blastocyst stage with no need for media change [6, 9]. Both single-step and sequential media are commercially available

and have their unique concentrations of energy substrates to support preimplantation embryonic development as well as clinical outcomes (Fig. 1) [10]. Many studies compare those two types of media, their effect on the embryological outcomes, clinical outcomes, aneuploidy, or even the live birth rate [11 - 15]. However, it was difficult to identify which media is superior due to many cofounders that may affect the results [10, 16]. Emerging from those main approaches, many protocols can be used for embryo culture such as (I) interrupted culture system with the sequential culture media by refreshing the embryos in new media on day 3, (II) daily refreshment system with sequential culture media, (III) uninterrupted culture system with no refreshments using single-step media, (III) interrupted culture system either by refreshing the embryos on day 3 or with daily refreshment using single-step media.

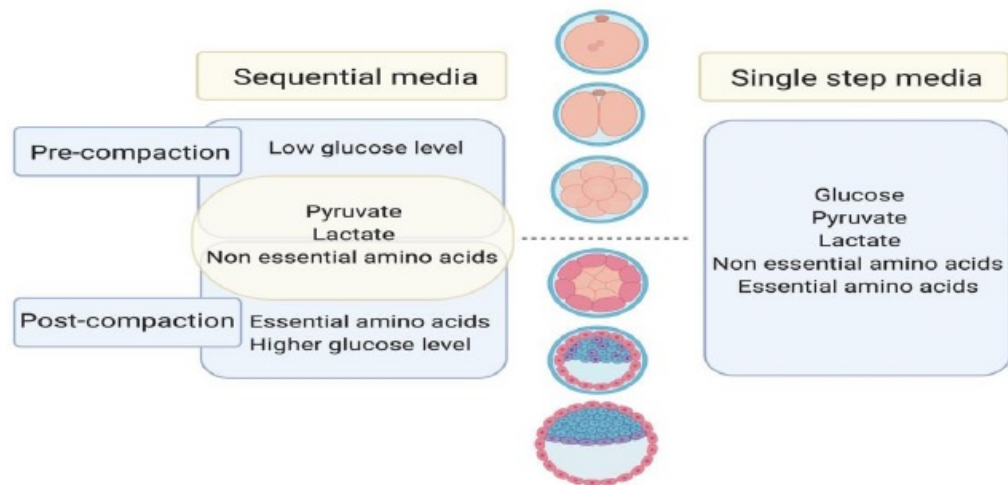


Fig. (1). Main energy substrates of a single step and sequential media for pre-compacting and post-compacting embryos.

Oxygen

The gas phase, especially oxygen is considered one of the most important factors for embryonic development [1, 17]. Oxygen has an important role in the energy production process, although the high concentration may lead to the formation of the reactive oxygen species, which had a toxic effect on gametes and embryos [18]. Its concentration within the uterus and oviduct is approximately 2%, 5-8%, respectively which differs from the oxygen concentration of the air 20% (Fig. 2) [17, 19]. In 1971, Wes Whitten found no blastocyst formation for mouse embryos cultured under 20% O₂, whereas 100% blastocyst formed under 5% O₂ [17]. He reported that excessive oxygen concentration may harm the surface membrane of the embryonic cells [17]. Also, in the case of human embryonic cells, different O₂

Assisted Hatching

Jayesh Parasharam Shinde^{1,*}

¹ ART Fertility Clinic PVT LTD, B 404 Aishwaryam Comfort, Akrudi, Pune-411019, Maharashtra, India

Abstract: The selected Spermatozoa when it reaches the ovulated Cumulus Oocyte Complex after ovulation, dispersion of the granulosa cells and corona radiata cells occur. The Spermatozoa then must cross the Zona Pellucida (ZP), fuse with the oolemma, and then subsequently fertilize the oocyte. Embryologist Karl Ernst von Baer coined the term 'Zona Pellucida' from Greek work Zone which means belt or girdle and Latin work Pellucida which means transparent or shining. This extracellular matrix is about 13-15 um thick and surrounds all the mammalian eggs and pre-implantation embryos. Zona Pellucida structure is made up of carbohydrates, specific proteins, glycoproteins, hyaluronic acid, heparin, collagen, and fibrous proteins. Human Zona Pellucida contains 4 glycosylated proteins namely ZP1, ZP2, ZP3, and ZP4. ZP plays an important role in helping oocytes to transport essential nutrients and helps in avoiding polyspermy by hardening after fertilization. The embryos must break open the protective ZP layer to the implant, the process is called hatching. It is said that in Assisted reproductive treatment (ART) factors such as the non-availability of enzymes from the endometrium which helps in hatching, extended culture, vitrification may lead to failure in the hatching of embryos from ZP. It was postulated that micromanipulation of ZP to create an opening will help the embryos to hatch and thus implant and will lead to an increase in Implantation rates (IR). This process was later called Assisted Hatching (AH). Various methods were discovered for Assisted hatching such as mechanical ZP AH, zona digestion using enzymes, and laser-Assisted hatching. This chapter will focus on the advantages and disadvantages of each method of AH and their applications in ART along with the impact of AH on clinical outcomes. The use of any method of AH should be chosen carefully to avoid damage to the embryo which will defy the whole purpose of application of AH. In any case, laser-assisted hatching is widely used for Pre- Implantation Genetic Testing (PGT) of the embryos as it is very safe if applied properly, convenient, easy to use, and faster compared to other methods of AH. Each laboratory should identify the correct time and stage at which application of AH is considered based on whether it is helping to improve clinical rates or not.

Keywords: Assisted hatching, Chemical hatching, Laser-assisted hatching, Mechanical hatching, Zona Drilling, Zona Pellucida.

* Corresponding author Jayesh Parasharam Shinde: ART Fertility Clinic PVT LTD, B 404 Aishwaryam Comfort, Akrudi, Pune- 411019. Maharashtra, India; Tel: +91 7875891475; E-mail: jayesh.shinde45@yahoo.com

INTRODUCTION

The mammalian female gamete-Oocyte is surrounded by cumulus and corona cells and is called Cumulus Oocyte Complex (COC). This COC is released during ovulation. When the selected spermatozoa, after passing through various barriers of the female reproductive tract, reaches and penetrates the ovulated COC the dispersion of tightly bound granulosa cells and corona radiata cells occurs. The spermatozoa then have to cross the Zona Pellucida (ZP) to fuse with the Oolemma and then subsequently fertilize the oocyte [1]. In 1827, Embryologist Karl Ernst von Baer adopted the word 'Zona Pellucida' from Greek work Zone- meaning belt or girdle and Latin word pellucida meaning transparent or shining while describing the Human egg. This thick extracellular matrix (ECM) which is about 13-15 μm in diameter, surrounds all the mammalian eggs and preimplantation embryos [1, 2].

The structure of ZP is made up of carbohydrates, ZP-specific proteins, and Glycoproteins. Zp also contains hyaluronic acid, heparin, chondroitin (proteoglycans) and collagen, laminins, elastins, and fibronectins (fibrous proteins). Most mammalian eggs have 3 glycosylated proteins, human ZP contains 4 glycosylated proteins, namely ZP1, ZP2, ZP3, and ZP4. All these ZP1-4 have a polypeptide chain which includes the ZP domain (ZPD). The ZPD has 2 subdomains ZP-N and ZP-C connected by the linker region, 8 conserved cysteine residues, and ~270 amino acids (aa) [1, 2].

The main events during fertilization are sperm-COC recognition, penetration of ZP, and sperm oocyte fusion. The interaction between ZP and sperm is species-specific by receptor-ligand interaction leading to activation of sperm. Molecules like murine tyrosine kinase and galactosyltransferase are believed to be the sperm's receptors for ZP. Glycoprotein ZP3 acts as a ligand for receptors present on the sperm surface. This binding of sperm results in exocytotic event releasing proteolytic enzyme which will facilitate the penetration of ZP by the sperm, the process is called Acrosome reaction, where ZP2 acts as secondary sperm binding site resulting in gamete interaction *via* contact of ZP with the inner membrane of sperm which has undergone Acrosome reaction [3]. Such a complex interaction and process may hint towards the indirect role of ZP in sperm selection. Post fertilization, exocytosis of cortical granule content in perivitelline space (PVS) happens and comes in contact with the oolemma and ZP. This exocytosis and cortical reaction lead to the blocking of multiple sperms entering the oocytes and thus avoiding polyspermy [4].

Zona hardening happens post-fertilization and is helpful to avoid polyspermy, protect the preimplantation embryo during early embryo development, and

blastomere integrity and passage of embryo in the female reproductive tract before implantation. It is also argued that ZP protects the embryos from various uterine factors and immune responses. Zona hardening is also observed in mouse and human embryos cultured *in vitro* [1, 5, 6].

For the embryo to implant, it must hatch out of its protective layer-ZP. Based on time-lapse observations it was believed that repeated expansions and contractions of the blastocyst help the embryo exert pressure on the ZP to assist its hatching and implant. The volume of the blastocyst increases thereby stretching the epithelium of trophoblast cells and exerting increasing hydrostatic pressure leading to thinning of ZP. The presence of specialized plump cells called 'Zona Breakers' are also found to be present near the opening of the ZP [7]. Now there are sufficient studies in the database which suggest that various enzymes, proteolysins such as trypsin, proteases, and uterolysins, which are secreted by either the blastocyst and/or endometrium are responsible for the hatching of the embryo [7, 8, 16].

ASSISTED HATCHING AND ART

There are many reasons which may lead to the failure of implantation of a viable embryo. Sometimes the embryo may be slow-growing or its inherent quality may not be good to implant. Endometrium may not provide adequate uterine factors to assist implantation or might not be receptive enough. Another reason might be due to the inability of the embryo to hatch owing to circumstances related to laboratory and clinical conditions [11]. Implantation of the embryo after hatching is based on a series of events that need to be completed in a coordinated manner. Failure in completing any one of these numerous steps might stop the embryo from implanting. Patients' ability to produce enough uterolysins may be diminished or the ability of such uterolysins to carry out their function may also be influenced due to assisted reproductive treatment cycles they are undertaking [13].

Improvement of culture conditions led to extended cultures in many IVF labs around the world to select good quality blastocyst and transfer fewer embryos. This might lead to compromise the hatching process as enzymes that are thought to be released by endometrium are present to assist the hatching in natural conception. Extended culture might also lead to disrupting the timing and mechanism of hatching as Zona hardening is observed [1, 7, 9, 13]. *In vitro* culture embryos are found to be slower in development or poor in quality or many of them may not reach the blastocyst stage or hatch when compared to the development of embryos in natural conditions [10]. FSH levels, pre-ovulatory oestradiol, cause of infertility, and advanced maternal age also have an impact on

CHAPTER 9**Oocytes and Embryos Cryopreservation****Mohamed Fadel El Mohr^{1,2,*} and Islam M. Saadeldin^{3,4,5,*}**¹ *Dr. Faris Medical Center for Infertility and Human Reproduction, Heliopolis, Cairo, Egypt*² *Dar El Om for Infertility and Human Reproduction, Mokattam, Cairo, Egypt*³ *Research Institute of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea*⁴ *College of Veterinary Medicine, Chungnam National University; 34134 Daejeon, Republic of Korea*⁵ *Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt*

Abstract: The availability of cryopreservation technology has extended the scope of human-assisted conception treatment and made it more convenient for patients. Freezing of oocytes and ovarian tissue is now taking place in research institutions and should hopefully become widely available for clinical use in the future if the women have to be away at a critical time during the treatment; the frozen oocyte was thawed and used when required. Good quality embryos frequently remain after the transfer of the required number into the woman; these can be frozen and used at a later date, if required, instead of the couple going through another cycle of in vitro fertilization (IVF) treatment. The conduct of cryopreservation in humans is based on information originally derived from animal work, where sperm cryopreservation, for example, has been carried out for several decades for animal breeding. This chapter will consider the principles of cryopreservation followed by a general description of the practical steps. The clinical applications of cryopreservation will also be described. A final section will deal with frozen embryo replacement.

Keywords: Oocyte and embryos cryopreservation, Slow freezing, Thawing embryos, Vitrification.

HISTORY OF OOCYTE AND EMBRYOS CRYOPRESERVATION

Early basic scientific advancements in measurements of temperature and therefore the chemistry of solutions and gases are certainly sentinel events for cryobiology. It's been suggested that original versions of the tools to live temperature were

* **Corresponding authors Mohamed Fadel El Mohr and Islam M. Saadeldin:** Dr. Faris Medical Center for Infertility and Human Reproduction, Heliopolis, Cairo, Egypt; Tel: +201006311052; E-mail: fadell20@gmail.com and Research Institute of Veterinary Medicine, Chungnam National University, 34134 Daejeon, South Korea; Tel: 00821024817666; E-mails: islamms@cnu.ac.kr and islamsaad82@gmail.com

made by Galileo within the early seventeenth century. The primary accurate means of measuring temperature were developed in the early 1700s by the German physicist Gabriel Fahrenheit through the application of mercury in glass.

Since then, modifications of instruments to assess temperature became significantly more accurate and easier to use. Equally important were early advancements made within the nineteenth century involving understandings of liquefaction of gases and the potential use of such refrigerants to chill and store specimens at extremely low temperatures. When one traces the history of mammalian gamete cryopreservation, numerous accounts reference the start of low-temperature biology to 1866, when an Italian military physician Mantegazza documented the observation that human spermatozoa became immotile when cooled in the ice [1, 2].

Investigations in the late 1940s and early 1950s by Chang [3, 4] on low-temperature storage of rabbit oocytes, zygotes, and embryos paved the way for studies on the cryopreservation of female gametes and embryos. Subsequent experiments by Sherman and Lin [5 - 8] demonstrated that mouse oocytes could even be cooled in glycerol, stored, and subsequently fertilized in recipients; furthermore, resulting in embryos supported pregnancies.

In the 1960s and early 1970s, a merging of basic/theoretical cryobiology and practical studies ultimately gave rise to the increased success of embryo cryopreservation. Classical basic science investigations by Mazur [9 - 11] formed the inspiration for understanding cell-specific optimal cooling and warming rates which today remain a pivotal key to successful mammalian gamete and embryo cryopreservation. It had been the combined strengths of Mazur, Leibo, and Whittingham that resulted in successful cryopreservation of mouse embryos.

The cryoprotective agent used by these researchers was 1.5 M dimethylsulfoxide (DMSO), which was combined with a slow cooling rate (0.3C/min to 80C) and deposited in liquid nitrogen [12]. Slow cooling rates supported dehydration during cooling and prevented intracellular ice formation, so this technique was used. In addition, it was discovered that the cryoprotective agent (here DMSO) should be added and removed in a stepwise manner to avoid osmotic shock or injury. In the early 1980s, the same cryopreservation methods were used to successfully create the first human pregnancies after freezing and thawing [13, 14].

Testart *et al.* pioneered the use of 1, 2-propanediol (PROH) as a permeating cryoprotectant for pronuclear-stage zygotes [15]. Furthermore, as an osmotic buffer, these researchers used sucrose in the cryo-media as a non-permeable cryoprotectant. Slow cooling rates were used until the temperature reached 30°C,

after which the samples were submerged in liquid nitrogen and the warming rate was rapid.

Cryopreserving pronuclear- and cleavage-stage mammalian embryos has become a popular practice in assisted reproductive technology (ART) labs throughout the United States and around the world. As experience with cryopreservation of different cell types grew, it became clear that as cell size grows, so does the complexity of cryopreservation [11].

This principle is particularly important in the cryopreservation of mammalian oocytes and embryos slow-rate freezing and vitrification are the two approaches currently used to cryopreserve mammalian oocytes and embryos [16].

Effects on oocyte and embryonic cellular functions may impair abilities to develop normally following the cryopreservation process, regardless of the cryopreservation method used. Oocyte and/or embryo “cryo-damage” is the collective term for these damaged cellular events although the effects of cryopreservation on documented and/or theoretically specific cellular structures and functions, as well as subsequent effects on oocyte and embryonic developmental competence, have been previously reviewed [17].

Cells are subjected to a variety of stresses during cryopreservation, including mechanical, thermal, and chemical stresses [11, 18], which can disrupt cell function and cause cell death. Oocytes, in general, are more vulnerable to cryo-damage than later embryonic stages moreover slow-rate freezing attempts to control biophysical properties of freezing, such as cooling and warming rates, in conjunction with cryoprotectants to minimize adverse cellular events. This approach allows cells to be cooled to extremely low temperatures while minimizing the production of intracellular ice crystals and attempting to avoid the negative effects of increased solute concentrations and osmotic stress [19]. As a result, extracellular ice formation drives cellular dehydration into an equilibrium mechanism when slow-rate freezing is used.

In recent years the new technique of vitrification came to avoid the mistakes of slow freezing, the word “vitrification” comes from the Latin word vitreous, which means glassy or similar to glass. Vitrification is a non-equilibrium cryopreservation method that was originally designed for the cryopreservation of mammalian sperm [20] and embryos [21]. This technique used a special device known as straw-like Fig. (1) and is divided into three regions 1st leaf which put embryos or oocytes on its 2nd plastic mark to write the name and id of the couple and 3rd the coverslip.

Reproductive Cloning

Eman A. Hussien¹, Sara A. Mekkawy¹, Elham K. Eltahawy², Islam M. Saadeldin^{3,4,5} and Mohamed M. Omran^{2,*}

¹ Biotechnology Program, Faculty of Science, Helwan University, Cairo, Egypt

² Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt

³ Research Institute of Veterinary Medicine, Chungnam National University, 34134 Daejeon, Republic of Korea

⁴ College of Veterinary Medicine, Chungnam National University; 34134 Daejeon, Republic of Korea

⁵ Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt

Abstract: Hearing the name “Dolly” was and still stirs the minds of professionals and non-specialists towards the term “cloning”, but the way of producing dolly is not the only aspect of cloning. Cloning is defined as the techniques through which identical or virtually identical individuals can be produced. Based on this definition, in this chapter, we are trying to clarify the different applications, aspects, and techniques of cloning such as gene cloning, therapeutic cloning, but to focus on reproductive cloning. Reproductive cloning is the method of making a genetically similar clone of a whole organism. Then it is needed to be discussed with all the scientific thoughts around it, advantages, disadvantages, legal or illegal, and comparing it to other aspects and this is our aim in this chapter.

Keywords: Cloning, Embryo splitting, Reproductive cloning, Somatic Cell Nuclear Transfer, Tetraploid complementation.

INTRODUCTION

The term “cloning” was and is still being widely used by biologists to express many concepts. Even though all these concepts depend on the usage of copies of the whole biological material, they vary in their precision. There are three common major applications of cloning; the first is gene cloning which means the cloning of DNA segments [1, 2], the second is cells cloning which is done to give rise to particular types of cells, and the third is individual cloning that seeks the

* **Corresponding author Mohamed Mostafa Omran:** Chemistry Department, Faculty of Science, Helwan University, Ain Helwan, 11795, Cairo, Egypt; Tel: 00201025585562; E-mails: drmmomran@science.helwan.edu.eg and drmmomran@yahoo.com

mimicking of the natural process in the identical twin development specifically in multicellular organisms Table 1. The first case to appear and to spread the concept of cloning to the public is the famously known case of Dolly, which was successfully produced by cloning after about 270 trials by the British scientist Ian Wilmut. After its success, trials have been done on many types of mammals such as mice, rats, pigs, sheep, cows, goats with an increasing success rate without reaching 100%. The spontaneous abortion was the fate of most of the cloning pregnancies [3]. In the case of fetus survival from death during pregnancies, if they did not face early death, they were seen to have serious health concerns such as distorted limbs, gross obesity, dysfunctional immune systems, and organs such as liver and kidneys, and also lungs and the progressive diseases and failures were one of the reasons of the early death of Dolly along with arthritis after living only 6 years after its development by cloning, causing its health to decay rapidly [4, 5]. With all these consequences, it was nearly impossible to apply to humans especially since there are more serious consequences that are abridged in devastating economic, health, and emotional ramifications due to the fetus's death [5].

Table 1. Different applications of cloning.

-	Gene Cloning (Molecular Cloning)	Therapeutic Cloning	Gene Cloning (Molecular Cloning)
Definition	The process of isolating a DNA sequence of interest to make multiple copies of it. The identical copies are clones	The technology used to produce tissues and organs from a single cell to replace damaged cells, tissues, and organs	The process of generating an organism that has the same DNA as another organism
Product of cloning	produces copies of genes or segments of DNA	produces embryonic stem cells of engineered tissues for experiments	produces copies of whole animals
Applications	<ol style="list-style-type: none"> Main aim: Studying the structure and function of genes in detail. Medical applications: the synthesis of vitamins, hormones, and antibiotics Agricultural applications: cloning in bacteria facilitate nitrogen fixation in plants 	<ol style="list-style-type: none"> Main aim: To make use of the unique ability to generate virtually all types of cells in an organism. Medical applications: Grow healthy tissues in the laboratory that can be used to replace damaged or diseased tissues. Learn more about the molecular causes of diseases. 	<ol style="list-style-type: none"> production of organs for transplantation to humans Studies of human genetic diseases. Basic research in the control of gene expression and function
Techniques	Recombinant DNA technology	Somatic cell nuclear transfer	<ol style="list-style-type: none"> Somatic cell nuclear transfer Embryo splitting

(Table 1) cont....

-	Gene Cloning (Molecular Cloning)	Therapeutic Cloning	Gene Cloning (Molecular Cloning)
Potential drawbacks	Nature is an extremely complex, interrelated chain. It is believed by some scientists that the introduction of genetically-modified genes may have an irreversible effect with consequences not yet known.	There are striking similarities between stem cells and cancer cells. Both cell types can proliferate indefinitely and some studies show that after 60 cycles of cell division, stem cells can accumulate mutations that could lead to cancer.	Reproductive cloning is a very inefficient technique and most cloned animal embryos cannot develop into healthy individuals. For instance, Dolly was the only clone to be born live out of a total of 277 cloned embryos. This very low efficiency, combined with safety concerns, presents a serious obstacle to the application of reproductive cloning.

The characteristics of clones are not only genetically but also environmentally controlled, the nutritional inputs during pregnancy or from parents after birth, society, or life experience while growing up and other elements can be determinants of the clone's characteristics. So, if there are two genetically identical clones as the nucleus and the mitochondria donor are the same but are grown in different environments or at different times, they will not be identical in their physical or behavioral characteristics. Even monozygotic twins are not fully genetically identical or epigenetically identical as many elements contribute to their variation such as mutations, stochastic developmental variations, and varied imprinting effects (parent-specific chemical marks on the DNA) that differ in impact on each twin [6, 7]. If twin clones do not have the same mitochondria, as there may be multiple donors or the cell is obtained from an individual and the nucleus is obtained from another, there are additional variations that will exist especially in the high energy demanding body parts such as the heart, muscles, brain, and eye. Also, some differences exist in the body systems that are controlled by mitochondria to determine cell numbers by using mitochondrial control over cell death [8 - 10].

HUMAN CLONING VS. ANIMAL CLONING

In the context of human cloning, there is no scientific evidence that human embryos have been cloned (Table 2). In 1998, there was a claim from South Korean scientists to be successful in cloning a human embryo till the phase of a four cells embryo, and then the experiment was interrupted. In 2002, a kind of religious group called Clonaid claimed the birth of a girl named eve to be the first cloned human and the first of another 12 cloned humans, but also without introducing any evidence to prove their claim. In 2004, a paper was published in

SUBJECT INDEX**A**

- Ability 24, 98, 224
 frozen-thawed ram semen fertilizing 98
 stem cell's 224
- Acetylcholine 24
- Acid 12, 17, 22, 23, 46, 72, 96, 100, 174, 175,
 180, 181, 187, 188
 citric 22, 23, 72, 100
 hyaluronic 12, 33, 174, 175
 lactic 17, 46
 oleic 96
 Tyrode's solution (ATS) 180, 181, 187, 188
- Acrosome reaction 12, 72, 175
- Actions 4, 45, 47, 51, 177
 anabolic 47
 blocking androgen 4
 luteotropic 51
 paracrine 45
 thermogenic 47
 uterolysin 177
- Activity 4, 14, 24, 25, 47, 55, 120, 145
 follicular 120
 growth-stimulating 55
 myometrial 47
 oscillatory 145
 parasympathetic 24, 25
 sympathetic 24, 25
- Adrenergic post-ganglionic fibers 35
- Albumin 14, 157, 207
 human serum 157, 207
 recombinant Human 207
- Aldosterone secretion 57
- Alkaline phosphatase 98
- Androgen(s) 5, 7, 13, 14, 17, 19, 25, 36, 42,
 44, 46
 adrenal 14
 binding protein (ABP) 13, 17
 granulosa 44
 producing 19
 receptors 5
- Aneuploidy screening 188
- Animal 93, 227, 234
 based products 93
 cloning efforts 234
 embryos 227
- Antibodies 54, 69, 70
 anti-sperm 70
 monoclonal 69
- Antiestrogens 118
- Antifreeze proteins 97
- Antimullerian hormone (AMH) 5, 7, 13, 112,
 120
- Anti-oxidizing agent 21
- Antral follicles 27, 28, 42
- Antrum, follicular 28
- AOA techniques 143, 144, 145, 146
- Apoptosis 7, 44, 78, 94, 96
 decreasing cell 94
- Application 147, 148, 149, 174, 183, 184, 185,
 186, 187, 188, 189, 190, 191, 218, 219,
 220, 224, 229
 ionophore 147, 148, 149
 medicinal 229
 reproductive 224
- Assay, enzyme-linked immune sorbent 79
- Asthenozoospermia 77, 81
- ATP synthase 95
- Atresia 29, 30, 32, 33, 113, 120
 follicular 120
- Autoimmune reactions 13

B

- Biochemical 72, 117
 components fructose 72
 hyperandrogenism 117
- Biomarkers 95, 99, 112
 freezability 99
 proteomic 95
- Biopsies 187, 188, 208
 blastocyst-stage 187
 trophectoderm 208

Subject Index

Blastocyst 51, 52, 53, 155, 176, 179, 180, 186,
187, 188, 207, 208, 224, 225, 226, 227,
228
biopsies 188
collapsed 186
embryos 208
formation 155
BMR and enhancing growth 47
Body mass index (BMI) 121
Bovine 95
 seminal ribonuclease protein 95
 spermatozoa alginate encapsulation 95
 sperm freezability 95
Brucellosis 126, 137, 138, 140
Bulbourethral gland lipase 96
Bull sperm 93, 94, 95
 cryopreservation 93, 94
 encapsulation 95
 freezing 94
 lyophilization 94

C

Calcium 57, 58, 145
 deposition 58
 homeostasis 57
 oscillations 145
Camel 99,100
 semen cryopreservation 99, 100
 sperm 100
Cameras 162, 165
 time-lapse 162
Cancer 117, 220
 endometrial 117
Capsule 8, 18
 vascular 8
CART analysis 99
CASA method 74
Cavity, healthy receptive endometrial 112,
122
Cell 77, 101
 debris 77
 population, abnormal sperm 101
Cellular 91, 128

Advances in Assisted Reproduction Technologies 255

 plasma membranes 91
 structural elements 128
Chemical transmitters 24
Chemotherapy 117, 120, 204
Chilling injury 201, 202
Chorionic 51, 53, 54
 endothelium 53
 gonadotropins 51, 54
 interstitium 53
 somatomammotropin (CS) 55
Chromatin condensation, normal 81
Chromosome(s) 4, 5, 10, 26, 62, 113, 159,
188, 191, 221, 227, 228
 dynamics 159
 genes-related sex 113
 haploid number of 10, 26
 matured mammalian oocyte 227
Clinical pregnancy rates (CPR) 181, 188, 189
Clitoris 7, 46
Cloning 218, 219, 220, 221, 224, 227, 228,
229, 230, 232, 233, 234, 235, 237, 238,
239, 240
 applying handmade 228
 artificial 232
 gene 218, 219, 220
 method 228
 nonreproductive 224
 therapeutic 218, 219, 220, 224, 240
Clostridia infections 137
Clotting enzymes 22, 23
Concentration 13, 50, 65, 66, 67, 68, 72, 154,
155, 156, 199, 201
 intracellular calcium ion 50
 nutrient 154
Congenital malformations 190
Connective tissue, vascular 20
Contractions 19, 22, 25, 46, 48, 176
 antiperistaltic 22
 rhythmic 46
Controlled zona dissection 179
Control 15, 40
 of reproductive cycle 40
 of testis functions 15
Conventional semen analysis 95
Corona radiata cells 174, 175

- Coronavirus 137
Corpus 36, 135
 albicans 36
 hemorrhagicum 36
 uteri 135
CPR in frozen embryo transfers 188
Cranial vagina 131, 132
Cryobiology 195, 205
Cryopreservation 90, 91, 93, 95, 96, 98, 99,
 100, 101, 186, 195, 196, 197, 199, 205,
 206, 209
 effect of 90, 91, 197
 injury 206
 mammalian gamete 196
 methods 196, 197
 process 185, 197
 technique 209
 technology 195
Cryopreserved sperm 95, 100
Cryoprotectant(s) 90, 92, 93, 100, 101, 129,
 130, 197, 199, 201, 202, 205, 206, 209
 agents 100, 199
 combinations 201
 milk-based 93
 toxicity 209
Cryoprotective 97, 196
 agent 196
 effect 97
Cryptolerance 90, 95
Cryptorchidism 18, 19
Cryptozoospermia 65, 148
Culture media 153, 154, 156, 157, 158, 161,
 162, 166, 168, 178, 181, 203
 commercial 154
 embryonic 154, 157, 161, 162
 supplementation 156
 utilization 163
Culture oil 161
Culture system 153, 155, 158, 167, 168
 dynamic 167
 interrupted 155
Cumulus oocyte complex (COC) 175
Cycles 25, 37, 39, 41, 42, 118, 119, 143, 156,
 177, 189, 190, 195, 220
 consecutive 143
 frozen 190
 ovarian 25, 39, 41
 ovulatory 118
 reproductive treatment 176
Cytosolic calcium deficiency 145
- ## D
- Damage 174, 178, 182, 185, 200, 206, 221,
 223
 lipid 206
Defective genetic profiles 221
Degeneration 181, 185
 cytoplasmic 181
De novo mutations 229
Density 66, 77, 120, 161
 baseline bone 120
Density gradient centrifugation (DGC) 77, 78,
 81, 86, 98, 101
 method 86
Dependency, photoperiod 139
Depolymerization 202, 206
 microtubule 206
Deposition 23, 46, 76
 glycogen 46
Development of ovarian follicles 28
Diagnosis, genetic 163
Dihydrotestosterone 5, 7, 14
Disease(s) 39, 92, 93, 117, 119, 120, 137, 140,
 204, 219, 221, 224, 225, 226, 227, 232
 autoimmune 225
 cardiovascular 117
 disorders 224
 genetic 119, 137, 226
 transmissions 92, 93
 transmitted 137
Disorders 112, 115, 117, 118, 119, 120, 137,
 138, 187, 188, 223, 224, 225
 autoimmune 120
 autosomal dominant 119
 epigenetic 223
 monogenic 188
 ovulatory 112, 115, 118
 prevalent hormonal 117

Subject Index

single gene 187
thyroid 120
Disruption, mechanical 190
Diversity 222, 231, 233, 239, 241
 genetic 222, 233
Divisions, mitotic 51
DNA 81,182, 218, 219, 220, 232
 integrity and morphology 81
 methylation 144, 229
 segments 218, 219
DNA damage 79, 101, 182
 oxidative 101
Dysfunction 82, 115, 117, 223
 ejaculatory 82
 immune 223
 ovulatory 117
Dysfunctional immune systems 219

E

Effects 17, 155, 182
 mutagenic 182
 of LH on Leydig Cells 17
 toxic 155
Egg plasma membranes 50
Electrolytes, intracellular 92
Electrophoresis 101
Elevated growth weight and high neonatal
 mortality 222
Embryo(s) 26, 154, 155, 158, 159, 162, 163,
 164, 165, 168, 174, 176, 177, 178, 179,
 180, 181, 182, 183, 184, 186, 187, 188,
 189, 190, 197, 200, 206, 208, 222, 223,
 227
 assessments 178
 biopsy 163, 188
 blastocyst stage 177, 184, 187
 choose theories 154
 cleavage stage 184, 185, 187
 cryo-preservation 206
 culture system 168
 density 166, 168
 destruction 222

Advances in Assisted Reproduction Technologies 257

development 26, 159, 163, 165, 176, 179,
 181, 184, 187, 190
frozen-thawed 189, 200
lysed 200
mammalian 227
morphology assessments 177
mosaic 188
post-compacting 155
single early-stage two-cell 223
storage 206, 208
tetraploid 227
thawing 195
transfer 130, 144, 162, 163, 166, 178, 188,
 207
trapping 185
waste 236
Embryo cryopreservation
 programs 206
 restrictions 204
Embryoblasts 52
Embryogenesis 8, 14, 138
 sustainable 138
Embryologists 60, 144, 146, 147, 149, 162,
 163, 185, 188
Embryonic 153, 154, 155, 156, 157, 158, 159,
 161, 164, 165, 166, 167, 168, 197, 230
 cellular functions 197
 development 153, 154, 155, 156, 157, 158,
 159, 161, 164, 165, 166, 167, 168
 stem cell process 230
Embryo splitting 218, 219, 223, 227, 241
 method 241
 process 223
Emotional ramifications 219
Encephalitis 117
Endocrine anomalies 72
Endometrial stroma proliferates 40
Endometritis 137
Endometrium 36, 39, 47, 52, 54, 55, 56, 174,
 176, 177, 179, 180, 184, 186
 necrosed 39
Endoplasmic reticulum 10, 11, 145
 smooth 145
Energy 55, 138, 147, 155
 production process 155

- Environmental 60, 221
 conditions 60
 habitats 221
- Enzymes 5, 7, 11, 12, 12, 13, 22, 24, 26, 45, 48, 50, 79, 94, 96, 145, 146, 149, 174, 176, 179
 acrosomal 50
 antioxidant 94
 cytoplasmic 26
 egg yolk-coagulating 96
 hydrolytic 11, 12
 linked immune sorbent assay (ELISA) 79
 lytic 45
 phospholipase 96
 pronase 179
 protease 179
- Eosin-Nigrosin assay 71
- Epididymis 3, 7, 8, 11, 19, 20, 21, 22, 61, 66, 83
- Epitheliochorial placenta 53
- Epithelium 2, 5, 40, 176
 coelomic 2, 5
 endometrial 53
- Estrogen 4, 13, 20, 25, 34, 35, 36, 37, 40, 42, 45, 46, 51, 53, 55, 56, 57
 synthesize 4, 13
- Estrogen 17, 38, 43, 46, 55, 57
 during pregnancy 55
 environment 44
 and progesterone 43, 46, 55, 57
 secretion 38, 55
 synthesis 17
- Estrus cycle and menstrual cycle 37
- Events of folliculogenesis 29
- Excess 137, 117
 insulin 117
- Excretory function 54
- Exocytosis 94, 175
- Exocytotic event 175
- Exotic agents 147
- Extraction 84, 223
 nuclear 223
 testicular epididymis sperm 84
- Extruded liposome 94
- F**
- Factors 33, 36, 37, 39, 40, 60, 118, 121, 134, 145, 157, 163, 168, 174, 176, 184, 189, 190, 201
 anti-apoptotic 157
 colony-stimulating 157
 embryo-trophic 163
 hormonal 134
 hypothalamic 40
 immunological 133
 leukemia inhibitory 157
 luteolytic 36, 37
 male fertility 60
 meiosis-inducing 33
 neuroendocrine 40
 sperm-derived oocyte activation 145
 uterine 176
- Fatty 43, 91, 96
 acids 43, 96
 acyl chains 91
- Feeding disorders 133
- Female(s) 7, 58, 125, 130
 oestrous 125, 130
 phenotype determination 7
 reproductive systems development 58
- Ferguson reflex 48
- Fertility 90, 93, 95, 96, 100, 101, 113, 125, 132, 135, 205, 221
 female 113
 post-thawing camel semen 100
 post-thawing semen 96
 post-thawing sperm 101
- Fertilization 21, 22, 23, 36, 37, 45, 46, 47, 48, 49, 50, 52, 143, 144, 145, 146, 148, 149
 capacity, normal oocyte 146
 deficiency 146
 failure 36, 143
 ovum 27
 problems 144, 148, 149
 process 143
- Fertilizing efficiency 12
- Fetal 54, 222, 229
 deaths 222

Subject Index

fibroblasts 229
hemoglobin 54
Fetus immunorejection 55
Fibrinogen 22
Fibrinolysin 23
Fibronectins 175
Field 78, 101, 146, 205
 electric 101
 electrical 146
 embryology 205
 magnetic 78
Filtration 79, 80, 81
 glass wool 79, 80, 81
Flagellum, developing 11
Flow cytometry 69, 79
Fluctuates 119, 120
 follicular function 119
Fluids 1, 11, 12, 52, 61, 66, 67, 79, 183, 186,
 200
 acidic 67
 density 79
 dynamics 79
Follicle(s) 13, 16, 17, 18, 27, 28, 29, 30, 32,
 33, 34, 40, 41, 42, 44, 45, 55, 112, 113,
 114, 115, 118, 119, 120
 atretic 28, 33
 emerging 32
 growth 34, 44
 ovulatory 33
 resting 29
 stimulating hormone (FSH) 13, 16, 17, 18,
 30, 32, 40, 41, 42, 44, 45, 55, 112, 113,
 114
 tertiary 28
Follicular 34, 45, 49, 55
 cells, ovarian 49
 development and ovulation 34
 growth 45, 55
Follicular development 32, 115
 ovarian 115
Folliculogenesis 27, 29, 35, 41, 44, 113, 114
Follistatin 46
Formation 91, 149
 gastrointestinal tract 149
 intracellular ice crystals 91

Advances in Assisted Reproduction Technologies 259

Freeze 99, 130
 drying of sperm 99
 thawing protocols 130
Freezing 90, 92, 93, 94, 95, 96, 97, 98, 100,
 101, 128, 129, 196, 197, 205, 209
 fibroblasts and epidermal cells 205
 of oocytes and ovarian tissue 195
Frozen 81, 95, 188, 189, 208
 embryo transfers 188, 189, 208
 semen fertility 95
 thawed ejaculate 81
FSH secretion 18, 42, 45, 46, 116
 regulating 45
 stimulated follicle growth 45
Function(s) 1, 5, 9, 13, 14, 20, 21, 46, 47, 55,
 56, 58, 113, 116, 120, 162, 219, 223
 disrupt cell 197
 gonadal 15, 116
 maternal immune 55
 mitochondrial 162
 of estrogen 46
 of progesterone 47
 ovarian 120
Fusion 49, 50, 145, 149, 175, 227
 sperm oocyte 175

G

Galactosyltransferase 175
Generation of embryonic stem 229
Genes 4, 5, 6, 97, 114, 117, 139, 219, 222,
 226, 228, 229
 autosomal 5
 encoding 5, 97
 lethal 139
 mitochondrial 228
 mutated 226
Genesis 238
Genetic 133, 144, 163, 240
 constellation 144
 defects 133
 innovations 240
 sequencing 163
Genomic integrity 229

- Germ cells 2, 3, 4, 5, 12, 13, 17, 26, 60, 69, 113
 diploid 5
 excessive immature 69
 immature 69
- Gestation 4, 26, 51, 55, 56, 113, 180, 226
- GH-like protein in humans and primates 55
- Glands 8, 21, 23, 24, 43, 52
 accessory genital 21
 accessory sex 8
 endometrial 52
 vaginal 24
- Glass wool filtration process 79
- Globozoospermia 65, 148, 150
- Glucocorticoids 57, 117
- Glycerol concentrations tolerability 92
- Glycerolisation 129, 135
- Glycerophosphocholine 12
- Glycogenolysis 46
- Glycoprotein(s) 5, 17, 48, 114, 174, 175
 disulfide-linked dimeric 17
 polymer 114
- Gonadotrophin 34, 116
 preparations 116
 signal 34
- Graafian follicle* 25, 27, 28, 33, 35, 47, 125
- Growth 14, 15, 29, 32, 42, 46, 47, 48, 53, 55, 57, 180, 222
 allantois 222
 alveolar 55
 hormone 57
- Growth factors (GFs) 113, 114, 154, 156, 157, 168, 184, 186
 fibroblast 157
 supplementation 156
 transforming 157
 vascular endothelial 157
- Guanylyl cyclase 24
- H**
- Hatching 176, 179, 180, 183, 184, 185
 blastocysts 185
 process 176, 179, 180, 184
- natural 183
- Head agglutination 70
- Health 116, 219, 234
 sexual 116
- Healthy breeding 125
- Heat 133, 137
 detection 133
 stress 137
- Hemospermia 65
- Hemochromatosis sarcoidosis 117
- Hepatitis 82, 203
 B virus (HBV) 203
 C virus (HCV) 82, 203
- High 17, 55
 intracellular testosterone levels 17
 levels of estrogen in late gestation 55
- Higher power's creation 240
- Histiocytosis 117
- Histone acetylation 229
- Honolulu technique 228
- Hormones 1, 2, 6, 13, 14, 35, 40, 42, 43, 45, 51, 54, 55, 56, 57, 58, 114, 132
 anabolic 14
 gonadotropin-releasing 35, 40
 hypothalamic 42, 43
 ovarian 40, 43, 45, 57
 ovulatory 132
 polypeptide 56
 testicular 7
- HOS test 71, 72
- Human 26, 101, 113, 161, 163, 176, 198, 204, 206, 219, 220, 221, 227, 230, 236, 240
 embryos 26, 113, 161, 163, 176, 220, 221, 230, 236, 240
 genetic diseases 219
 oocytes 198, 204, 206, 227
 sperm cell suspension 101
- Human cloning 220, 221, 222, 232, 233, 235, 237
 critics 235
- Hyaluronidase 12
- Hydrated living cells 209
- Hydroallantois 222
- Hyperinsulinemia 118
- Hypermotility 75

Subject Index

Hyperosmotic shock 91
Hyperprolactinemia 115, 122
Hypersecretion 118
Hyperspermia 65, 66
Hypocalcemia 137
Hypoestrogenism 119
Hypogonadism 116
Hypogonadotropic hypogonadism (HH) 115, 117, 122
Hypo-osmotic swelling test 71
Hypospermia 65, 66, 81
Hypothalamic-hypophysial-portal circulation 15

I

Immotile sperms 20, 68, 70, 74, 77, 81
Immunogenic responses 229
Immunoglobulins 79
Immunosuppression 225
Implantation process 52
Implanting blastocyst 51, 52
Incubator technologies 165
Infant mortality 189
Infections 66, 67, 69, 72, 83, 117, 137, 223
 accessory genital gland 72
Infectious anemia 127
Inflammation 69, 133
Influence FSH secretion 46
Inhibin 16, 17, 18, 22, 32, 40, 42, 43, 44, 45, 46
 plasma 17
Inhibin hormone 13, 17
Insemination(s) 86, 126, 131, 140
 laparotomic 140
 method 86
 techniques 126, 131
Interaction 157, 175
 embryonic 157
 receptor-ligand 175
Intrafollicular modulator 45
Intra-testicular vascular elements 18
Intrauterine Insemination and skin health 137
Ionization process 162

Advances in Assisted Reproduction Technologies 261

Ionomycin 143, 148

K

Kallmann syndrome 117
Kartagener syndrome 148
Karyotyping 120
KPIs embryologists 144

L

Lactogenesis process 57
LAH and acid tyrode 179
Laparoscopic ovarian drilling 119
Laser 66, 99, 101, 128, 174, 179, 181, 182, 183, 185, 186, 187, 188, 191
 assisted hatching (LAH) 174, 179, 181, 182, 183, 185, 186, 187, 188, 191
 energy 182
 pulses 182
Lateral sclerosis 227
Lecithin 93, 96
Legitimate human responsibility 237
Leptospirosis 137
Leucosis 137
Leydig and sertoli cells 5
Life-threatening diseases 230
Light microscopy (LM) 180
Lipid peroxidation 94
Liposomes 94
Liquid 201
 homogeneous watery 66
 nitrogen 99, 101, 128, 196, 197, 198, 201, 203
Live sperms 71
Livestock breeding 130
Lou Gehrig's disease 227
Low-density lipoproteins (LDL) 93, 97
 lyophilized 97
Lungs 1, 136, 219, 223
 fetal 223
Luteinizing hormone (LH) 14, 15, 16, 17, 32, 33, 34, 35, 37, 40, 42, 43, 44, 116, 121
Luteogenesis 113, 114

Luteolysis 36, 37, 42, 48, 51, 55
Luteolytic effects 36
Luteotropic factor 36, 37, 51
Lyophilization 94
Lysins 177
 enzymatic 177
Lysolecithin 96

M

Magnetic activated cell sorting (MACS) 78, 81
Male(s) 7, 18, 19, 60, 63, 64, 74, 82, 148
 cryptorchid 19
 infertility 60, 63, 64, 74, 82, 148
 normal domestic 18
 phenotype determination 7
Malformation 149
Mammalian 9, 27, 146, 197, 230
 oocytes 146, 197, 230
 ovaries 27
 Testis 9
Mammary gland development 57
Maternal 48, 51, 53
 cytoplasmic inheritance 53
 recognition of pregnancy 48, 51
Maturation, follicular 121
Mature oocyte 25, 45, 187, 188, 227
Mechanical hatching 174
Mechanism(s) 19, 26, 30, 49
 of follicle deviation 30
 of oogenesis 26
 of ovulation 49
 thermoregulatory 19
Medically assisted reproduction (MAR) 60, 64, 79, 82, 86
Medications 65, 121
 oral ovulation 121
Meiotic division 10
Meiotic spindles 158, 200, 202
 functional 202
Melatonin 35
 effect 35
Menopause 39, 113, 114

Menstrual cycle 37, 38, 39, 113, 116
Mesenchymal stromal cells 2
Mesenchyme cells 7
Metabolic 14, 47, 55, 57
 effects 14
 rate 47, 55, 57
Metabolism 25, 158, 159, 177
 cellular 159
 embryonic 158
Mineral oil (MO) 148, 161, 181
Mitosis 4, 113, 145, 148
Mitotic germ cell multiplication, rapid 113
Monozygotic twinning 185, 189
Motile spermatozoa 61, 74, 77, 79, 83
 virus-free 83
Motile spermatozoa sticking 70
Multiple pregnancies 115, 118, 119, 121, 122, 156, 206
Mural granulosa cells nearest 28
Murine tyrosine kinase 175
Myometrial contractions 55, 56

N

Natural process 219
Nature 22, 55, 79, 154, 165, 166, 167, 220, 227, 232, 235, 236, 238
 diploid 227
 dynamic 166, 167
 mimic 165
Necrosis 36, 39
 tumor 36
Necrozoospermia 65
Neonatal 149, 222, 229, 233
 deaths 233
 health issues 222
Nerves 24, 112
 internal pudendal 24
 pelvic 24
Nervous system malignancies 117
Neurons 24, 25, 35, 43, 205
 hypothalamic 43
 postganglionic 24
Next generation sequencing (NGS) 188

Subject Index

Nitric oxide 24
Nitrogen 165, 219
 fixation 219
 gas 165
Non 36, 62, 166
 gravid uterus 36
 humid incubators 166
 spermicidal condom 62
Non-sperm 66, 67, 70, 83
 cells 66, 67, 70, 83
 elements 70
Normal 19, 62
 human sperm 62
 spermatozoa, producing 19
Normozoospermia 65, 81
Nuclear 229
 reprogramming efficiency 229
 transfer embryos 229
Nutrition 53
 hematotropic 53
 nutrition-placental 53
Nutrition during embryonic development 53
Nutritive function 54

O

Obstruction 67, 72, 75
 epididymal 72
 potential ejaculatory duct 72
Oestrus synchronisation 130, 132, 135
Oil 67, 97, 148, 160, 161, 162, 167, 181
 coconut 97
 crude 161
 immersion 67
 mineral 148, 161, 181
 palm 97
 paraffin 161
 synthetic 161
Oligomenorrhea 116
Oligoovulation 115, 122
Oligoteratozoospermia 64
Oligozoospermia 65
Ongoing pregnancy rates (OPR) 188

Advances in Assisted Reproduction Technologies 263

Oocyte(s) 1, 12, 27, 50, 114, 145, 146, 159,
 175, 185, 195, 197, 198, 201, 203, 205,
 206, 228
 activation 145, 146
 cryopreservation process 205
 cytoskeleton 159
 plasma membrane 50
Oogenesis 25, 26
 mammalian 26
 process 25
Osmolality 122, 158, 159, 160, 161, 166
 proper embryo culture media 159
Osmotic shock 196
Osteoblasts 47
Osteoporosis 119
Ovarian 27, 28, 29, 30, 31, 33, 38, 45, 112,
 113, 114, 115, 121, 122, 184, 190, 209
 follicles 27, 28, 29, 30, 31, 33, 38, 45, 112,
 113, 114
 hyperstimulation 115
 reserve 112, 113, 121
 stimulation 112, 115, 116, 121, 122, 184,
 190, 209
Ovulation 25, 26, 27, 28, 30, 33, 34, 35, 38,
 40, 42, 47, 112, 113, 114, 115, 117, 118,
 119, 122, 127, 134
 disorders 115
 induction 112, 115, 118, 119
 inhibin 42
 obesity hinders monitoring 117
 periodic 34
 process 47, 112, 114, 115
 stimulation 122
Oxygen 54, 137, 155, 156, 223
 concentrations 155, 156
 therapy 223
Oxytocin 22, 36, 43, 44, 46, 48
 secretion 43
 synthesize 36

P

Panagiotidis 207
Paraffin oil (PO) 161

- Pathway 37
 - local utero-ovarian 37
 - systemic 37
- PCOS women 118
- Pelvic irradiation 204
- Penis, gland 24
- Percutaneous epididymal sperm aspiration (PESA) 84
- Peroxidation reactions 161
- Phospholipase 144
- Pinocytosis 54
- Pituitary 7, 16, 17, 18, 35, 37, 42, 43, 57, 116
 - FSH secretion 18
 - gland 7, 35
 - gonadotropes 16
 - gonadotropins 17, 43, 57
 - hormones 42, 57
 - regulation 116
- Placental 55, 56, 57
 - lactogen (PL) 55, 57
 - trophoblast 56
- Plant-based cryoprotectants 93
- Plastic polymers 164
- Polycystic ovary syndrome (PCOS) 115, 116, 117, 118, 119, 121, 122
- Polydimethylsiloxane chips 100
- Polyspermy 12, 49, 50, 145, 175
- Polyzoospermia 65
- Pregnancy 36, 39, 47, 48, 51, 54, 55, 56, 57, 112, 122, 133, 134, 138, 141, 148, 153, 222
 - abnormal 222
 - detection 133, 134
 - Index 141
- Preimplantation genetic testing (PGT) 166, 174, 183, 187, 191
- Premature ovarian insufficiency PCOS 117
- Preovulatory follicle 28, 45
- Pressure 22, 23, 24, 159, 176, 183
 - intracellular 183
 - osmotic 23, 159
- Primary ovarian insufficiency (POI) 115, 119, 120, 122
- Primordial 9, 27, 28, 114, 229
 - follicles 27, 28, 114
 - germ cells (PGCs) 9, 229
- Problems 66, 116, 138, 145, 203, 205, 209, 221, 224, 228, 232, 233, 234
 - dysfunctional 116
 - hypothalamic-related amenorrhea 116
 - microscopic 138
 - oocyte-related 145
- Procedure 62, 63, 84
 - microscopic 63
 - semenology 62
 - semen processing 84
 - sperm wash 83
- Process 9, 10, 11, 12, 29, 30, 36, 113, 114, 115, 174, 175, 187, 199, 219
- Processing of surgically retrieved sperms 83
- Production 8, 15, 16, 23, 46, 47, 51, 52, 94, 96, 113, 130, 132, 227, 230, 241
 - agricultural 130
 - commercial semen 132
 - fatty acid 96
 - uterine milk 52
- Profibrinolysin 23
 - prostatic 23
- Progesterone 36, 39, 40, 41, 42, 43, 45, 46, 47, 52, 55, 56, 57, 113
 - luteal 42
 - plasma 42
 - production 36, 45, 113
 - secretion 56
- Progressive sperms 68
- Prostaglandins 22, 62, 130
- Prostate gland 8, 22, 66
- Proteins 5, 13, 14, 45, 55, 57, 91, 92, 95, 97, 154, 156, 157, 174, 175, 221, 225
 - anabolism 14
 - caprine heat shock 97
 - divergent mitochondrial 225
 - fibrous 174, 175
 - hormones 45, 55
 - glycosylated 174, 175
 - sperm equatorial segment 95
 - synthesis 57, 154
- Proteoglycans 33, 175
 - synthesize 33
- Proteolysins 176

Subject Index

Proteolytic enzyme 62, 66, 179
Pulmonary hypertension 223
Putative roles 157
Pyospermia 65

R

Radiotherapy 117, 120
Ram semen cryopreservation 97
 protocols 97
Ram's post-thawing acrosome integrity 97
Reaction 50, 122
 acrosomal 50
 inflammatory 122
 reverse transcriptase-polymerase chain 83
Reactive oxygen species (ROS) 69, 79, 92,
 155, 162
Recovery, post-partum 138
Regular vaccination programs 137
Regulates FSH and LH secretion 41
Release metabolites 185
Renal agenesis 117
Reproductive 1, 130, 205, 241, 233
 biotechnology 130
 cloning activities, prohibiting 241
 process 233
 techniques 1, 205
Reproductive cloning 227, 230, 231
 applications 231
 methods 227, 230
 techniques 223
 technology 231
Risk 92, 93, 116, 117, 118, 119, 120, 121,
 122, 183, 185, 190, 203, 224, 225, 236
 cardiovascular 119
 hypothetical 203
 long-term cardio-metabolic 116
Role of steroid hormones in implantation 52
ROS production and mitochondrial 99
 membrane 99

S

SCNT 223

Advances in Assisted Reproduction Technologies 265

 method 223
 process 223
Screening, genetic 208
Secondary oocyte 25, 27, 33, 35, 50
 releasing mature 25
Secretions 7, 13, 14, 16, 21, 22, 23, 36, 38, 40,
 45, 46, 53, 56, 57, 58, 118
 acidic vaginal 46
 gonadotropin 40
 hepatic insulin 118
 maternal growth hormone 57
 seminal vesicle 22
 uterine 53
 uterine milk 56
 vaginal 22, 23
Segregates sperms 81
Semen 24, 25, 61, 74, 95, 96, 97, 128, 131
 analysis by CASA method 74
 cryopreservation 96, 97
 freezability 97
 freeze-thawing 128
 preparation methods 61
 low freezability 95
 propulsion of 24, 25
 transferring 131
Seminal 23, 61, 66
 fluid's resistance 66
 neutral glucosidase 61
 vesicle fibrinogen 23
Seminal plasma 21, 72, 74, 75, 77, 79, 93, 95,
 96, 97, 98
 proteins 93, 95
Severe Oligoasthenoteratozoospermia (SOAT)
 64
Single nucleotide polymorphism (SNPs) 97
Sinus, urogenital 7, 14
Skimmed milk 98
Solid Surface Vitrification (SSV) 101
Somatic cells nuclear transfer (SCNT) 223,
 224, 228, 230, 241
Spermateliosis 9
Spermatoc cord 8, 19
Spermatids 10, 12, 13, 17
Spermatocytes 13
Spermatocytogenesis 9

- Spermatogenesis 9, 10, 12, 16, 17, 69, 72
- Spermatogenic 9
 - cells 9
 - Process 9
- Spermatogonia lining 9
- Spermatozoa 7, 8, 9, 11, 12, 17, 18, 20, 21, 22, 61, 71, 72, 74, 76, 77, 78, 79, 91, 94, 186
 - abnormal 77
 - apoptotic 78
 - bovine 94
 - dehydration 91
 - immotile 79
 - live 61
 - plasma membrane 91
- Sperm(s) 11, 12, 13, 21, 22, 48, 50, 60, 61, 65, 68, 70, 76, 81, 90, 92, 93, 95, 96, 99, 100, 101, 144, 146, 175, 195, 235
 - apoptotic 81
 - bovine 93, 95
 - buck's 96
 - cryopreservation 90, 92, 100, 195
 - cryptolerance 91, 93
 - donation 146, 235
 - filtration 96
 - maturation 11
 - migration based methods 76
 - washing 96
- Sperm cell(s) 62, 79, 90, 91, 92, 93, 94, 97, 99, 101, 128, 129
 - bovine 93
 - dehydration 97
 - immature 62
 - immotile 79
- Spermiogenesis 9, 10, 11
- Sperm preparation 60, 75, 79, 80
 - method 60
 - techniques 75, 79, 80
- Sperm cytoplasm 21, 77
 - defective 77
- Sperm encapsulation 95
 - freezing sex-sorted 95
- Sperm freezability 94, 95
 - lyophilized 94
- Sperm lyophilization 11, 94, 99, 197
 - mammalian 11, 197
- Sperm segregation 80, 83
 - virus-free 83
- Stallion 99, 139
 - spermatozoa 139
 - sperm freezability 99
- Stem cells 21, 219, 220, 221, 224, 225, 227, 229
 - blastocyst-derived embryonic 229
 - embryonic 219, 224, 225, 229
 - pluripotent 229
 - skin 225
 - therapeutic transplantation 224
- Steroidogenesis 14, 44, 46
- Steroidogenic Factor I (SFI) 5
- Stresses 69, 71, 80, 92, 117, 129, 154, 158, 159, 187, 197, 238, 239
 - chemical 197
 - mechanical 158
 - metabolic 187
 - moderate hypo-osmotic 71
 - osmotic 92, 197
 - oxidative 69, 80, 129
 - thermal 159
- Surgical sperm retrieval methods 83
- Syndrome 115, 116, 117, 119, 120, 121, 122, 204, 209, 222
 - metabolic 117
 - ovarian hyperstimulation 121, 122, 209
 - ovary hyperstimulation 204
 - polycystic ovary 115, 116, 117, 122
- Synthesis 5, 14, 15, 40, 44, 219
 - erythropoietin 15
- Synthetic progestogens 130
- System 1, 8, 12, 23, 164, 167, 181, 224, 226, 234
 - dynamic 167
 - immune 12, 224, 226, 234
 - mouth-controlled suction 181
 - osmotic pressure buffering 23

Subject Index

T

Technologies 73, 74, 90, 95, 101, 126, 130, 165, 166, 219, 222
 artificial intelligence 101
Teratozoospermia 65, 77, 81
Test 71, 79, 112, 120, 164, 186, 187
 sperm immobilization 79
Testicular epididymis sperm aspiration (TESA) 84
Testosterone 5, 7, 9, 13, 14, 16, 17, 22
 and dihydrotestosterone 7
 plasma 16
Tetraploid complementation method 241
Therapies 118, 119, 132, 204, 224, 225
 antibiotic 132
 antiestrogen 118
 gonadotrophin 118, 119
Thermolysis 182
Thermotaxis 96
Tissues 12, 7, 24, 200, 209, 219, 224, 227, 228
 cavernous 24
 embryonic 227
 engineered 219
 extraembryonic 227
 frozen 228
 interstitial 12
Tray agglutination test (TAT) 79
Turner syndrome 119

U

Ultrasound 112, 117, 119, 120, 122, 133
 pelvic 120
 transvaginal 112
Uterolysins 176

V

Vapor-phase systems 203
Vasoactive intestinal peptide (VIP) 24
Virus, human immune deficiency 203

Advances in Assisted Reproduction Technologies 267

W

Wegener's granulomatosis 117
WHO guidelines 60, 69, 71

X

X-linked disorders 119

Z

Zona 144, 145
 protein 144
 reaction 145
Zygote nutrition 47



Islam M. Saadeldin

Dr. Islam M. Saadeldin obtained his Ph.D. degree from the Seoul National University, South Korea. He worked as a postdoctoral researcher at Seoul National University, a visiting scholar at Niigata University, Japan, and an associate professor at King Saud University, Saudi Arabia. Currently, he is a research professor at Chungnam National University, South Korea. He has a patent of invention regarding bovine embryo transgenesis through PiggyBac transposons and he authored more than 160 research papers, reviews, and book chapters covering the fields of advanced reproductive biotechnology such as somatic cell nuclear transfer (SCNT), transgenesis, adult and embryonic stem cells, as well as elucidating the roles of extracellular vesicles in embryo communication and the embryonic-maternal crosstalk. His current research focuses on developing CRISPR/Cas9 engineered extracellular vesicles for improving embryo implantation and pregnancy of cloned and transgenic animals. He has been awarded several national and international prizes, such as the Egyptian State Prize, Shoman Prize, Almarai Prize, Misr Elkheir prize, The Interstellar Initiative for young investigators, the International Embryo Technology Society (IETS) Early Career Achievement Award, and AUA Scholar Award.