

Towards Improving the Results of Assisted Reproductive Technology (ART)

Essay

Submitted in partial fulfillment of master degree

In Obstetrics and Gynecology

By

Dalia Mahmoud Aly Shaheen

M.B. B.Ch.

Ain Shams University

Supervised By:

Prof. Dr. / Hisham Sayed Badawy

Professor of Obstetrics and Gynecology

Faculty of Medicine

Cairo University

Prof. Dr. Mohamed Mahmoud Waly

Professor of Obstetrics and Gynecology

Faculty of Medicine

Cairo University

Dr. Nawara Mohammed Hasheesh

Lecturer of Obstetrics and Gynecology

Faculty of Medicine

Cairo University

**Cairo University
Faculty of Medicine
2010**

Abstract

Assisted reproductive technology (ART) is by definition any treatment or procedure that includes the handling of oocytes and sperm or embryos outside the body for the purpose of establishing a pregnancy.

IVF was initially designed to overcome the problem of tubal infertility but is now widely held to represent the treatment of choice for unexplained infertility, male factor, endometriosis, and ovarian dysfunction resistant to ovulation induction.

ICSI is the ultimate option to treat these cases of severe male-factor infertility.

In 1990, preimplantation genetic diagnosis (PGD) was introduced as an experimental procedure to genetically screen human embryos during an IVF cycle. More than a decade later, PGD has become an established clinical procedure in ART.

The main advantages of this technique include storage for future use without repeating ovarian stimulation, chance of fertility for neoplastic patients who are going to receive chemotherapy, low risk of multiple pregnancies by reducing the number of fresh embryos transferred, and low risk, without canceling the cycle, of developing ovarian hyperstimulation syndrome.

Keywords:

In Vitro Fertilization, Intracytoplasmic Sperm Injection, Oocyte Retrieval, Assisted Hatching, Embryo Transfer, Preimplantation Genetic Diagnosis and Embryo Cryopreservation.

Acknowledgment

Thanks are given to **Allah** the source of all knowledge, by whose abundant aid this work has been accomplished.

It has been a great honor to do this work under the supervision of **Prof. Dr. Hisham El-Sayed Badawy** Professor of Obstetrics and Gynecology, Faculty of Medicine, Cairo University. I am greatly indebted to him for suggesting planning the subject and supervising the whole work. I will never forget his unlimited help, continuous support, kind encouragement and wise guidance.

I would like also to express my sincere gratitude and appreciation to **Prof. Dr. Mohammed Mahmoud Wally** Professor of Obstetrics and Gynecology, Faculty of Medicine, Cairo University for his valuable suggestions and assistance in this study. He has given me much of his time and experience, and without his help and effort, this work would have been impossible.

I would like to express my deep obligation to **Dr. Nawara Mohammed Hasheesh** Lecturer of Obstetrics and Gynecology, Faculty of Medicine, Cairo University for her useful assistance, great support and enlightening supervision, guidance, valuable advice and generous help in this work. To her I shall be forever grateful.

Finally, I must acknowledge the importance of my family. I am grateful to my parents who always believed that I could achieve something and provided unconditional love.

Dalia Mahmoud Aly Shaheen

Contents

	<i>Page</i>
List of Abbreviations	-
List of Figures	--
Assisted Reproductive Technology	1
Patient's Selection for ART.....	3
In Vitro Fertilization	15
Intracytoplasmic Sperm Injection (ICSI)	32
Oocyte Retrieval	35
Intracytoplasmic Sperm Injection (ICSI) (Micromanipulation)	42
Assisted Hatching	51
Embryo Transfer	58
Preimplantation Genetic Diagnosis	68
Embryo Cryopreservation	76
Summary	83
References	86
Arabic summary	---

List of Abbreviations

AA	: Ascorbic Acid
ART	: Assisted Reproductive Technology
APS	: Antiphospholipid Syndrome
ET	: Embryo Transfer
E2	: Estrogen
FDA	: Food And Drug Administration
FISH	: Fluorescent In Situ Hybridization
FRET	: Frozen Embryo Transfer
FSH	: Follicle-Stimulating Hormone
GIFT	: Gamete Intrafallopian Transfer
GnRH	: Gonadotrophin Releasing Hormone
GTN	: Glycerol Trinitrate
GV	: Germinal Vesicle
hCG	: Human Chorionic Gonadotrophin
HLA	: Human Leukocyte Antigen
HMG	: Human Menopausal Gonadotrophin
HSG	: Hysterosalpingography
ICM	: Inner Cell Mass
ICSI	: Intracytoplasmic Sperm Injection
IM	: Intramuscular
IUI	: Intrauterine Insemination
IVF	: In Vitro Fertilization

List of Abbreviations (Cont.)

LH	: Luteinizing Hormone
MESA	: Microsurgical Epididymal Sperm Aspiration
NO	: Nitric Oxide
OPU	: Oocyte Pick Up
OHSS	: Ovarian Hyperstimulation Syndrome
PCB	: Paracervical Block
PCR	: Polymerase Chain Reaction
PGD	: Preimplantation Genetic Diagnosis
PVP	: Polyvinylpyrrolidone
PZD	: Partial Zona Dissection
P4	: Progesterone
RCT	: Randomized Controlled Trials
RM	: Repeated Miscarriages
SSS	: Synthetic Serum Substitute
SUZI	: Subzonal Insemination
TESE	: Testicular Sperm Extraction
TET	: Tubal Embryo Transfer
TMC	: Total Motile Sperm Count
TVOR	: Transvaginal Ultrasound-Guided Oocyte Retrieval
uNK	: Uterine Natural Killer
WGA	: Whole Genome Amplification
ZIFT	: Zygote Intrafallopian Transfer
2-PN	: Two Clearly Visible Pronuclei

List of Figurers

<i>Fig.</i>	<i>Subject</i>	<i>Page</i>
1	Gross picture of hydrosalpinx	5
2	A representation of the luteal-phase commencement long down-regulation protocol using FSH for ovarian stimulation for assisted reproductive technology.	20
3	A diagram of the procedures, monitoring, and medicines used for short or Boost protocol commencing on day 2 of the menstrual cycle.	22
4	Schematic illustration of transvaginal ultrasound-guided oocyte retrieval	37
5	Oocyte maturity after cumulus and corona cell removal	45
6	Intracytoplasmic sperm injection procedure	47
7	Fertilization outcome after intracytoplasmic sperm injection	48
8	Embryo cleavage after intracytoplasmic sperm injection	49
9	Measuring the uterocervical angle prior to embryo transfer	64



Introduction

Assisted Reproductive Technology

Introduction

Assisted reproductive technology (ART) is any treatment or procedure that includes the handling of oocytes and sperm or embryos outside the body for the purpose of establishing a pregnancy. The first IVF baby was conceived in 1978 (Louise Brown) in Lancashire, England. IVF has been used in the United States since 1981 (***Von Wald and Thornton, 2007***).

The first birth in 1978 following in vitro fertilization (IVF) treatment marked the beginning of a rapid expansion of treatment modalities available to infertile couples as well as an improvement in success rates. IVF and many other assisted conception treatments are now routinely carried out in clinical practice and are proving to be more efficient and cost effective than some traditional medical remedies and tubal surgery. Conventional IVF is the name given to the original ‘test tube baby’ treatment method and involves addition of a measured volume of prepared sperm suspension to a dish containing the retrieved oocytes. Most other variants of conventional IVF still share several similarities with it. Where they differ is mainly in the method used to procure fertilization of the oocytes and the site of deposition of the gametes or embryos in the female genital tract (***Meniru, 2004***).

ART includes methods for assisted fertilization by intracytoplasmic sperm injection (ICSI) using sperm isolated from the ejaculate or obtained by microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE), assisted embryo hatching, and preimplantation genetic diagnosis (***Andersen and Erb, 2006***).

Other forms of ART involve the tubal transfer of oocytes and sperm (gamete intrafallopian transfer; GIFT), zygotes (zygote

intrafallopian transfer; ZIFT) or embryos (tubal embryo transfer; TET) via laparoscopy (*Basso and Olsen, 2005*).



Patient Selection for ART

Patient Selection for ART

Introduction

IVF was initially designed to overcome the problem of tubal infertility but is now widely held to represent the treatment of choice for unexplained infertility, male factor, endometriosis, and ovarian dysfunction resistant to ovulation induction (*Templeton et al., 2006*).

The introduction of ICSI has rendered severe forms of male infertility amenable to treatment and further widened the scope of IVF. High profile publicity given to the latest achievements with IVF has led to its perception as a panacea for all those having difficulty in conceiving a pregnancy. This has been reflected in the rapid expansion of indications for IVF and an estimated current annual number of IVF cycles worldwide approaching 500000, resulting in 1 in 100 to 1 in 150 babies born in the Western world being conceived by IVF (*Van Steirteghem et al., 2006*).

The degree to which IVF merits this growth in application remains unclear, however, since prospective randomized trials comparing the effectiveness of IVF with simpler fertility treatments are still lacking. Despite the relative lack of data objectively supporting the use of IVF, it has attained a central role in the treatment of infertility throughout the world. In recent years, data have emerged which allow us to re-evaluate how to assess these indications. It has become clear that factors other than the cause of infertility itself are of importance in determining the chance of success from IVF (*Macklon et al., 2005*).

Diagnosis as the indication for IVF

The original indication for IVF, tubal disease, remains an important indication for IVF. In terms of numbers of patients treated, however, other indications have become more important. This is reflected in the similar frequency of indications revealed by independent databases.

Although some account is taken of the woman's age, these indications are primarily diagnosis based (*Gardner et al., 2005*).

Male factor infertility has become a major indication for IVF. In the context of IVF this encompasses both qualitative and quantitative sperm abnormalities. Both sperm motility and morphology are considered to be important for determining IVF outcome in this group. The impact of motility on fertilization is corrected by the use of ICSI. Although ICSI has transformed the fertility prognosis for couples with severe male factor infertility, the appropriate indications for ICSI remain controversial. However, absolute indications for ICSI are agreed to include the use of microsurgical (epididymal or testicular) aspirated spermatozoa (*Hamberger et al., 2008*).

IVF indication list: (*Macklon et al., 2005*)

1. Tubal pathology

- If tubal surgery is not a realistic option, IVF is the method of choice.
- In case of impaired tubal function but no occlusion present, or after tubal surgery, IVF is the method of choice after an infertility duration of 2 years or longer. Depending on the woman's age IVF can be done after a shorter duration of infertility.

Hydrosalpinx

Ascending infection by microorganisms reaches the lumen of the fallopian tube and leads to inflammatory changes, leading to increased vascularity, exudation, and eventually destruction of the mucus membrane lining of the fallopian tube. Many of these infections, especially due to Chlamydia, are asymptomatic and the tubal damage might have occurred before these patients receive proper treatment. The end result will be destruction of the lining of the fallopian tubes; therefore, the ciliary cells

will be absent from many parts of the lumen of the fallopian tube. The fimbriated end becomes agglutinated, and the secretions in the tube will lead to distention and thinning of the wall of the tube, resulting in the formation of hydrosalpinx (*Patton et al., 1999*).

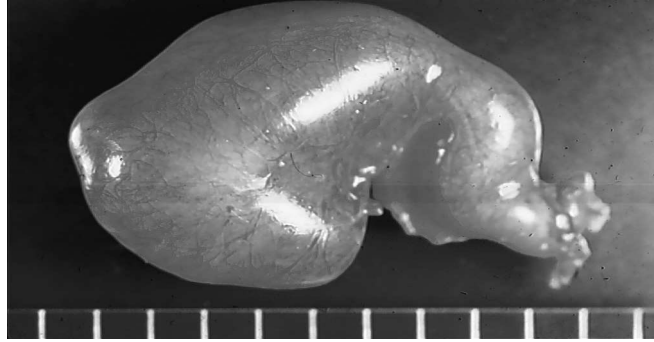


Fig (1):Gross picture of hydrosalpinx (*Badawy et al., 2008*).

Hysterosalpingogram studies in patients with hydrosalpinx reveals characteristic picture in which the tube is obstructed distally and distended with the dye. It is very interesting to note that the normal rugae of tubal lumen that are present in a normal fallopian tube are completely absent, and this suggests a poor prognosis for the fallopian tube if neosalpingostomy is done to correct this type of tubal obstruction (*Badawy et al., 2008*).

Several studies have found approximately a 50% reduction in the rate of delivery in women with a hydrosalpinx compared with women with tubal disease without a hydrosalpinx. The success rate increases to normal after tubal repair or salpingectomy. Endometrial integrin is reduced in many patients with hydrosalpinx and reverts to a normal pattern after salpingectomy. Occlusion of the proximal tube seems to be equally efficacious. Spontaneous pregnancy can occur when a unilateral hydrosalpinx is removed or repaired (*Shelton et al., 2006*).

Laparoscopic Removal of Hydrosalpinges

The presence of communicating hydrosalpinx was evidently shown to have a deleterious effect on fertility and IVF outcome. This is due to

the fact that such embryo toxic fluid keeps seeping into the endometrial cavity, leading to reduced endometrial receptivity, diminished embryo survivability, improper implantation, and increased incidence of abortion. Many studies have demonstrated significant improvement in the implantation rate and live birth rate in IVF patients after the removal of hydrosalpinx. According to the *Practice Committee of the American Society for Reproductive Medicine (2006)*, hydrosalpinx should be removed before the commencement of ART treatment to improve the implantation, pregnancy, and live birth rate.

Although the presence of hydrosalpinx could be diagnosed with ultrasound and HSG, these tests have relatively low sensitivity and specificity when compared with laparoscopy with chromopertubation, which is considered the golden standard for diagnosis (*Spielvogel et al., 2000*).

2. Male infertility

- Total motile sperm count (TMC) <1 million: first treatment of choice is ICSI.
- TMC >1 and <10 million: IVF can be performed if infertility duration is 2 years or longer.
- TMC >10 million: treat as unexplained infertility.

3. Unexplained infertility (idiopathic)

- In case of idiopathic infertility IVF is indicated if the duration is 3 years or longer. If the woman is older than 36 years, IVF may be considered earlier.

4. Endometriosis

Endometriosis is a common pathological process that mostly occurs in the female pelvis. Despite a long history of clinical experience and research, the pathogenesis and management of endometriosis still have a lot of uncertainty. Endometriosis is one of the most common