IN VITRO FERTILIZATION AND EMBRYO TRANSFER

THESIS

Submitted in Partial Fulfilment
of Master Degree
In

(OBSTETRICS & GYNAECOLOGY)

By

HATEM SAAD ISMAIL SHALABY
M.B.B.Ch.

UNDER THE SUPERVISION OF

Professor Dr. EL-SAYED EL-MAHGOOB
Professor of Obstetrics & Gynecology
Ain Shams University

Dr. ALI FARID MOHAMED ALI
Lecturer of Obstetrics and Gynecology
Ain Shams University

Faculty of Medicine
Ain Shams University

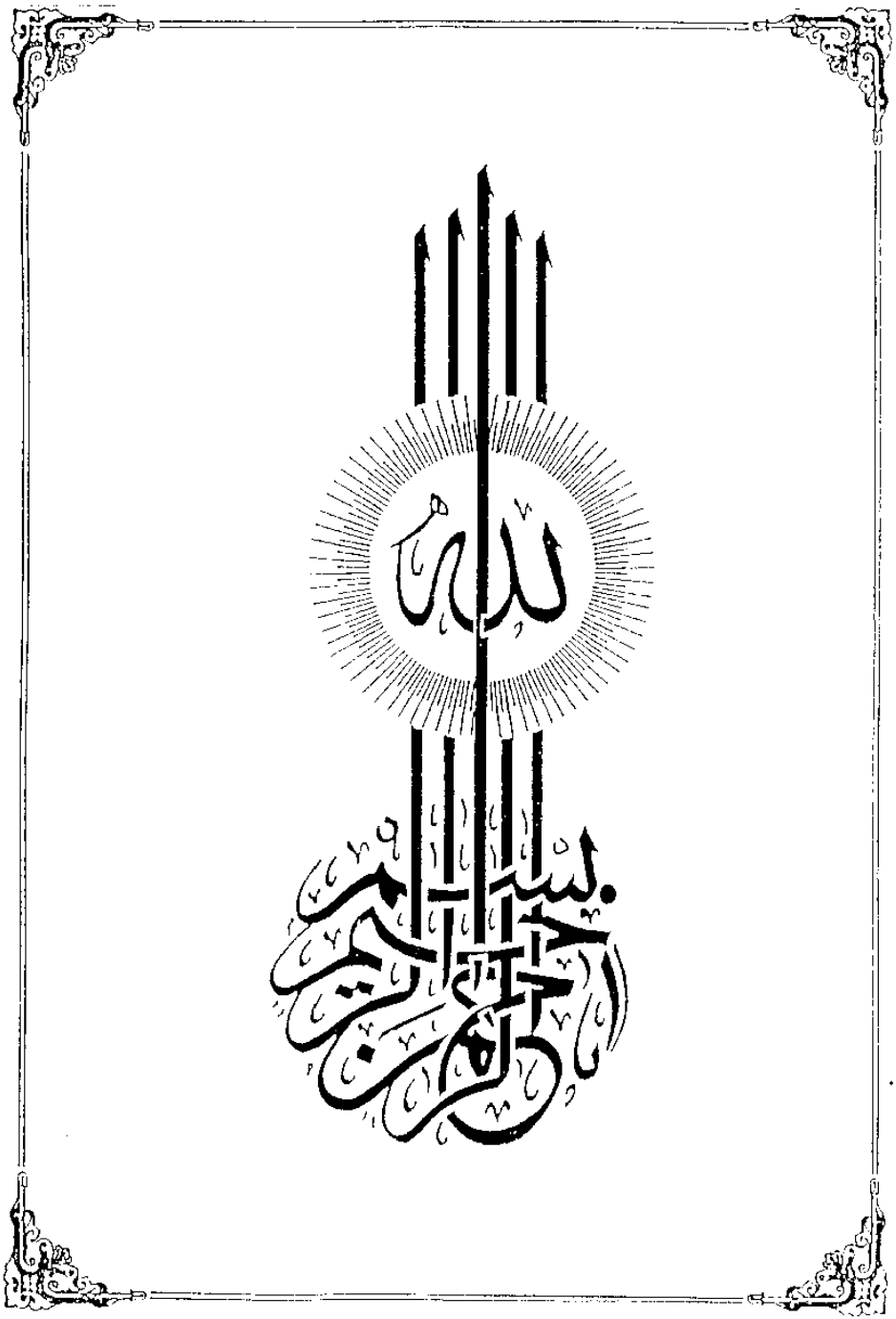
1986

ACKNOWLEDGEMENT

Appreciation is expressed to Professor SAYED EL MAGHOOB Professor of Obstetrics and Gynecology for his great interest and kind supervision and great support which made the completion of this work possible.

I wish to express my deep appreciation to Dr. ALI FARID Lecturer of Obstetrics and Gynecology for his supervision and helpful guidance.

I wish to thank Professor MOHAMED EL-MARAGHY, Dr. KARIM HASSANIN and Dr. ALAA EL-GINDY for multiple references they have supplied me.



C O N T E N T S

	Page
INTRODUCTION	1
AIM OF THE WORK	3
HISTORY OF INVTRO FERTILIZATION AND EMBRYO TRANSFER	4
INDICATIONS OF IN VITRO FERTILIZATION AND EMBRYO TRANSFER	9
SELECTION OF PATIENTS	19
PREPARATION OF PATIENTS	30
TREATMENT CYCLE	39
COLLECTION OF MATURE OOCYTES	68
OOCYTE CULTURE	95
ASSESSMENT AND PREPARATION OF SEMEN FOR IN VITRO FERTILIZATION	98
EMBRYO TRANSFER	126
QUALITY CONTROL PROCEDURE	155
MONITORING OF AN ESTABLISHED PREGNANCY	160
ETHICS OF IVF	182
SUMMARY AND CONCLUSIONS	190
REFERENCES	194
ARABIC SUMMARY	

* * *

I N T R O D U C T I O N

Man is entering a new era as a result of advances in human reproduction. Techniques have been developed to assist in the creation of man-artificial insemination and now in vitro fertilization (IVF) Soon. other new methods, based upon current advances of IVF procedure will develop to improve the quality of human reproduction. This work describes the conceptual framework and details of technique concerned with in vitro fertilization and embryo replacement (ER) or embryo transfer (ET).

In Vitro fertilization and embryo transfer is now a practical method of treating certain types of infertility. Its development has far greater implications opening new fields such as:

a) freezing of eggs or embryos,

b) donation of eggs,

c) transfer of sexed embryos for sex-linked diseases,

d) embryo microsurgery whic may enable infertile (non-motile) male spermatozoa to be used to produce normal offspring.

e) cloning by replication of some individual a number of times,

f) genetic engineering by insertion of foreign DNA which may protect the resulting offspring against disease or replace defective genes which result in genetic disease and lastly

g) transplantation of embryonic cells and tissues e.g. by transplantation of pancreatic tissue of fetal origin. Aborted fetal tissues may be more readily available for this procedure (Wood and Trounson 1984).

AIM OF THE WORK

The aim of the work is to review all aspects of in vitro fertilization and embryo transfer.

HISTORY OF IN VITRO FERTILIZATION AND EMBRYO TRANSFER

(History of in vitro fertilization and embryo transfer had passed into different era).

In the Mid-Nineteenth Century Tylor Smith had tried catheterization of the oviduct (1849) and various attempts of in vitro fertilization of mammalian ova had been tried during this period.

With the turn of century Morris in U.S.A. (1895) and Frank in Germany (1898) garfted ovarian tissue into the uterus on oviduct, an operation which was introduced primarily to treat symptoms arising from loss of internal secretion of ovary. Ests' operation (1909-1920) with rate of pregnancy 11% however fertilization and early cleavage in the uterine cavity is questioned (Biggers 1979 Adams 1979).

With mid-twenteith century; The in vitro fertilization prior to Edwards and Steptoe:

In animal field Lewis and Hartman have isolated a fertilized monkey ovum. In 1959 Hammond showed that it was possible to culture early mouse embryo from eight cell stage to the blastocyst in complex biological medium.

A few years latter, Whitten (1956) demonstrated that mouse embryo could develop from the two cell stage to the blastocyst in a simple medium. The following year, Mclaren and Biggers showed that mouse blastocysts produced by Whitten'sprocedure would develop into normal fertile adult mice after transfer into the uteri of foster mothers (Biggers 1981). Although many claims of successful IVF of mammalian eggs have been made over the years, it was not until 1959 that this event was unequivocally proved by Chang in rabbits by means of genetic markers (Biggers 1983).

In human field, the idea of human IVF and ET was suggested by Pincus since 1934 (Biggers 1981). In (1944) Rock and Kenkin reported in "Science" that they had isolated more than 800 human follicular eggs from surgical material and of these, 138 were observed after exposure to spermatozoa washed in Locke's solution. The eggs were usually cultured in fresh human serum. After 22 to 27 hours, two eggs were described as being in the two-cell stage and two in the three-cell stage.

Epilogue-Edwards and Steptoe and In vitro Fertilization, Edwards began his work on the human egg (1963) and he has collaborated with Steptoe since (1968) in which laparoscope was introduced to collect human oocytes from

Graffian follicles (Stepto and Edwards 1970) following numerous attempts the first conception in human was an ectopic pregnancy. On August 12, 1978, in a letter to the editor of lancet Steptoe and Edwards reported the birth of an infant girl weighing 2700 gm. "Pregnancy was established after laparoscopic recovery of an oocyte on Nov. 10 1977, in vitro fertilization and normal cleavage in culture media and the re-emplantation of the 8 cell embryo into the uterus 2½ days later". That is birth of Louise Joy Brown in Oldham General Hospital on July 25, 1978. The second birth (another girl), resulted by a similar procedure but with additional technological step (embryo freeze preservation) occurred in Calcatta, India on Oct. 3 1978 (Tayaraman 1978). The third birth (a boy), occurred in Edinburgh, Scotland on Jan. 14, 1979 by Steptoe and Edwards (Biggers 1981). The first twin, a female (Amanda) and a male (Steven) was delivered through the efforts of Trounson and Wood (Trounson et al., 1981).

To date, and in so far as the available information is accurate, the children born through IVF and ET procedures are apparently developing normally (Souport 1980). At first successful pregnancies in human resulted from transfer of 8-cell and 16-cell stage embryos fertilized in vitro (Edwards et al., 1980; Lopata 1980).

Although it is not made clear in their publications, apparently most of the embryos transferred by Edwards and Steptoe before the successful transfers were more advanced than 8-cell, mostly morulae and blastocysts.(Edwards et al., 1980).

In United States, human IVF research has been active in the early 1970s but come to standstill when regulations issued by the Department of Health and Welfare (HEW) were published in Federal Register of August 8. 1975 (Brachett and Seitz 1971; Soupart and Strong, 1975).

Too many obstetricians besides Edwards and Steptoe are interested in IVF and ET. A group headed by Professor Ronald Taylor, a group headed by Professor Ian Craft, and another group headed by Professor Beelin, in London. An Indian team working in Calcutta (Tayaraman 1978). An Australian team at Monash University headed by Carl Wood and Alan Trounson reported in IVF and ET as early as 1973. According to soupart personal experience the scope Australian team's scientific approach to IVF and ET is currently the broad cast (Soupart 1980). An American team headed by Professor Richard Blandou in Houston, Texas and another group headed by Professor Howard Jones in Virginia. There is a team working in Austria and in Italy headed by Professor Abatee.

Most of the current IVF and ET programs are university-based. The establishment of successful ambulatory IVF program in association with a busy, two-man general obstetrics-gynaecologic practice is reported with pregnancy rate with IVF and ET which Basically consists of Semenology laboratory, a fully equiped ambulatory surgical facility and an ultrasound unit. A local clinical chemistry laboratory provides daily plasma estradiol -measurements (Wood and Trounson 1984) with rapidly changing scientific as well as social environment the IVF and ET is subjected to many revolutions.

INDICATIONS OF IN VITRO FERTILIZATION AND EMBRYO TRANSFER

The technique of in vitro fertilization (IVF) and embryo transfer was first applied for the treatment of tubal infertility. Currently it is also being used for treatment of infertility due to other causes.

1. Tubal infertility:

Tubal infertility is defined as persistent bilateral tubal obstruction, absence of tubes or damage which has not produced bilateral obstruction but has resulted in a period of infertility of more than 24 months. The traditional treatment of tubal infertility is reparative surgery, but the success rates of operations range from 1-70% (Siegler 1960; Winston 1977, Gommel 1980). According to the nature of the operation and selection of the patients for the procedure. The overall success rate for tubal repairs even with the use of microsurgical technique is not higher than 30-50% and in the most favourable condition, only 75% success rate can be achieved (Wood and Trounson, 1982). Surgery has little or no chance of success in many patients with severe tubal disease (Gommel 1980).

Even if microsurgery is not successful, any adhesolysis achieved may improve the accessibelity of ovaries for laparoscopy and IVF (Wood and Trounson 1984).

The other alternative methods, mentioned below for treating tubal infertility have not met with general success (Trounson and Wood 1981). Replacing the tube by a graft has been attempted, but so far without success. While microvascular techniques are available which give a reasonable chance of techniqual success, the problems associated with finding suitable histocompatible donors and the hazards of anti-immune therapy are current barriers to further progress in tubal transplantation.

A silastic artificial Fallopian tube has been developed but the one surgical attempt to use it failed (Wood et al., 1971). A device which can replace both anatomical and physiological functions of the tube has not been made.

Attempts to overcome tubal infertility by transportion the ovary to the uterus as in Estes' operation (Estes and Heitmeyer 1954), have been occasionally successful, but the risk of the ovarian damage and uterine rupture, should pregnancy occur, have dettered surgeons from performing the operation. Analogous to this operation, is the transfer of mature oocyte from ovarian follicle to the uterine cavity, insemination by previous coitus or by the coincident transfer of washed sperm with the oocyte. Recently, experience by transfer of oocyte to the uterine cavity by Shettles et al. (1980), has reported two

pregnancies in seven patients. However, the technique used to obtain mature oocytes, the failure to identify oocyte in the follicular aspirate, the volume fluid (5-10 ml). transferred to the uterine cavity and the possible influence of coincidental tubal surgery would argue strongly against success of this procedure.

Aspiration of an ovum from the pre-ovulatory follicle and its low tubal ovum transfer in monkeys with surgically induced ampullary tubal obstruction can achieve in vivo fertilization and its inherent efficiency.

This method may avert numerous biologic and ethical problems confronting the use of in vitro fertilization and transfer of human embryos (Kreitmann and Hodge 1980).

Adoption is limited in many counteries and does not enable the patients to make a genetic contribution to the offspring, while surrogate pregnancy is accompanied by possible difficulties.