Ain Shams University
Faculty of Science

Practical Applications for Some Solvents as A Protecting Agent in Human Embryos Freezing Procedure during Assisted Reproductive Technology

A thesis submitted for the fulfillment of Master Degree of Science in Analytical Chemistry

BY

Norhan M.S.H. El. Saadany

B. Sc. Of Biochemistry and Chemistry (2003)
Faculty of Science
Ain shams University

2012

Practical Applications for Some Solvents as A Protecting Agent in Human Embryos Freezing Procedure during Assisted Reproductive Technology

BY

Norhan M. S. El. Saadany

B. Sc. Of Biochemistry and Chemistry (2003)

This thesis submitted for the fulfillment of Master Degree of Science in Analytical Chemistry and has been approved by:

Prof. Dr. Mostafa M. H. Khalil ……..

Professor of Analytical Chemistry
Inorganic and Analytical Chemistry Department
Faculty of science, Ain shams University

Prof. Dr. Mohamed M. Farrag ……

Professor and Chairman of Obstetrics and Gynecology Department, Director of IVF Unit
Faculty of Medicine, Suez Canal University

Date of examination: 19 / 7 / 2012

Head of Chemistry Department
Prof. Dr. Maged Shafik Antonious Nakhla

This thesis has not been previously submitted for any degree at this or any other university.

Norhan Mohamed Salah El. Dein Hussin El. Saadany

Acknowledgement

First of all, great thanks and praise to ***Allah*** for giving me prosperity and strength to fulfill this work. Deep gratitude and special thanks are devoted to my dear husband Dr. Mohamed El Aily for his patience, care and support.

All appreciation and dept are to ***Prof. Dr. Mostafa Mohamed Hassan Khalil***; Professor of Analytical Chemistry, Inorganic and Analytical Chemistry Department, Faculty of science, Ain shams University, for his valuable supervising, encouragement and sincere help through all stages of the study.

All gratitude and thanks are to ***Prof. Dr. Mohamed M. Farrag***; Professor and Chairman of Obstetrics and Gynecology Department, Director of IVF Unit, Faculty of Medicine, Suez Canal University, for his much appreciated supervising, effective guidance, offering facilities and fruitful discussions in all steps of this study.

Special gratitude and acknowledgement are for ***Dr. Waleed Ali Sayed Ahmad*** for following up the details and his great help during the experimental work.

Special gratitude and acknowledgement are for ***Dr. Elham Hussien Madany*** for her great guidance, support and help during the all stages of this work.

Many thanks are to the Chairman, Professors, doctors and staff members of Chemistry Department, Faculty of Science, Ain Shams University, and faculty of medicine, Suez Canal University

Contents

List of figures

List of tables

Nomenclature

ANOVA	Analysis Of Variance
ART	Assisted Reproductive Technology
CPA	Cryoprotective Additive
DMSO	Dimethylsulfoxide
DSC	Differential Scanning Calorimeter
ES	Equilibrium Solution
ET	Embryo Transfer
FSH	Follicular Stimulating Hormone
F-test	Fisher test (ANOVA F-test)
HCG	Human Chorionic Gonadotropin hormone
HSV	High Security Vitrification Straw
ICSI	Intracytoplasmic Sperm Injection
IVF	In Vitro Fertilization
IUI	Intrauterine Insemination
LH	Luteinizing Hormone
LN_2	Liquid Nitrogen
OPU	Ovum Pick Up
POH	1, 2 Propandiol
P- Value	Probability value
TB	Testicular Biopsy
VS	Vitrification Solution
WHO	World Health Organization

ABSTRACT

Abstract

Name: Norhan Mohamed Salah el. Dien Hussin El. Saadany
Title: Practical Applications for Some Solvents as A Protecting Agent in Human Embryos Freezing Procedure during Assisted Reproductive Technology.

The study is to assess the mode of action of some solvents as a protecting agent during human embryos freezing (cryopreservation, vitrification). Survival rate of human embryos had been assessed after vitrification process using two different freezing (vitrification) media with two different solvents; Dimethylsulfoxide (DMSO) based medium and 1, 2 Propandiol (PROH) based medium. This two solvents act as cryoprotective additives (CPA) that provide a protective effect for human embryos vitrification. Day2 or day3 divided human embryos were exposed to DMSO or PROH based medium before plunging into Liquid Nitrogen (LN_2) for long term storage. 212 embryos in vitrification process were split into two groups. Group A (114 embryos) were vitrified using sequential vitrification medium based on DMSO and Group B (98 embryos) were vitrified using sequential vitrification medium based on PROH. In the Warming procedure (Thawing) embryos of the group A and B were thawed respectively. Thawed embryos were transferred to physiological media designed for cleavage stage embryos and kept in the CO2 incubator for 2 to 8 hours. The survival rate of embryos was evaluated. After vitrification/thawing procedure, the survival rates of group A (DMSO) and group B (PROH) were 85 survived embryos of 114 (74.6%) and 52 survived embryos of

98 (52%) respectively (P.value<0.05). DMSO based medium used for vitrification/thaw process of day 2 or day 3 divided embryos demonstrated a significant higher embryo survival rate than that of PROH based vitrification medium. Results had been analyzed chemically to illustrate the mode of action of DMSO and POH and to explain the superiority of DMSO over POH.

Keywords:

Embryo cryopreservation, Vitrification, Dimethylsulfoxide; DMSO; 1,2-Propandiol; POH;.

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE S

I. Introduction and Review of Literatures

I.1. Infertility

There is no standard definition of infertility but biologically, inability of a couple to conceive and produce a live birth can be classified as infertility [**Healy, Trounson and Andersen; 1994]**. During their reproductive lives, 10% to 15% of couples are unable to achieve conception and deliver a living child after 1 year of unprotected coitus [**Healy DL, et al; 1994** & **Speroff, Glass and Kase; 1999]**.

Indications for early referral to a specialist fertility clinic can be summarized as:

1. Duration of infertility > 3 years.
2. Woman's age > 38 years.
3. Follicle stimulating hormone (FSH) concentration in early follicular phase > 10 IU/l.
4. Luteinising hormone (LH) concentration in early follicular phase > 10 IU/l.
5. Abnormal seminal fluid analysis as described by World Health Organization (WHO) 1999, Sperm count < 20×10^6/ml, Sperm motility < 50% motile, < 25%