

## *Acknowledgement*

*At the end of this work, I would like to say that I was honored by working under the supervision of Prof. Dr. Mohamed Abdel-Kader Al-Khafif, Professor of Clinical Biochemistry, Institute of Environmental Studies and Research Ain Shams University.*

*Words always failed to express how much I am deeply grateful to Professor Mohamed Abdel-Adl Elsayy, Professor of Human Genetics Departments of Pediatrics and Head of Genetics unit, Faculty of Medicine, Ain Shams University, for his fruitful advices and kind support while preparing this work.*

*Also, I would like to express my extreme gratitude to Dr. Sahar Mohamed Nour Eldin, Assistant Consultant of Pediatrics, Medical Genetics Center, Ain Shams University who guide me through every step of this work, she always there when I need help and giving me her time kindly. I am really impressed by her kindness.*

# List of Contents

Title	Page
List of Abbreviations	i
List of Tables	ii
List of Figures	III
Introduction	1
Aim of the present work	3
Review of literature	4
Human Development	4
Recurrent abortion	6
A-Definitions	6
B- Incidence	9
Etiological Factors of spontaneous abortion	11
1- Genetic Factors	13
2-Anatomic Factors	24
3-Endocrinal Factors	26
4-Immunological Factors	29
5-Teratogenic Factors:	31
A-Infectious Agent	32
B-Physical Agent.	41
C-Drugs	44
D-Chemical agents	51
5- Parental Factors	56
Evaluation and treatment for recurrent pregnancy loss	61
Subjects and Method	62
Results	70
Discussion	99
Summary	114
Conclusion and Recommendation	117
References	119
الملخص العربى	I

## **List of Abbreviations**

RPL	Recurrent Pregnancy Loss
hCG	Human chorionic gonadotropin
LMP	Last menstrual period
apL	Antiphospholipid
TORCH	Toxoplasmosis, rubella, cytomegalovirus and herpes
CMV	Cytomegalovirus
ETS	Environmental Tobacco Smoke
ISCN	International System for human cytogenetic Nomenclature
G-banding	Giemsa Banding
CNS	Central Nervous System
PCOS	Polycystic ovarian syndrome
ELIZA	Enzyme linked Immunosorbent Assay
DES	Diethyl stillbosterol

## ***List of Tables***

<b>Table</b>	<b>Title</b>	<b>Page</b>
1	Pregnancy loss classification	7
2	Classification of the etiology of recurrent spontaneous abortion.	10
3	Potential etiological factors in the causation of recurrent pregnancy loss	12
4	Types of chromosomal abnormalities	15
5	Evaluation and treatment for factors known to predispose to recurrent pregnancy loss	61
6	Descriptive statistics of the age groups of maternal and paternal age	70
7	Mean age and range in both males and females.	71
8	Comparison between cases with high and low number of abortions as regards maternal and paternal age	71
9	Correlation coefficient between maternal, paternal age and the number of repeated abortions among studied patients	72
10	Classification of cases as regards to the number of repeated abortions	73
11	The number of abortions in studied couples	74

## II

12	The number of abortions according to women's age	74
13	The number of abortions according to Men's age	75
14	Comparison between cases with low and high abortions as regards residence	76
15	Comparison between cases with low and high abortions as regard to <i>environmental exposures</i> near residence place:	77
16	Comparison between cases with low and high abortions as regards indoor air pollution	78
17	Comparison between cases with low and high abortions as regards to TORCH infections	79
18	Frequency of positive IgM and IgG against Toxoplasma, Rubella and CMV	80
19	Comparison between cases with high and low number of abortions as regards maternal and paternal intake of caffeine	80
20	Comparison between cases with low and high abortions as regards smoking of husband	81
21	Comparison between cases with low and high abortions as regards the job of the husband	82
22	Classifications of cases as regards semen analysis abnormality	83

### III

23	Comparison between cases with low and high abortions as regards abnormality of semen analysis	83
24	Comparison between cases with low and high abortions as regards to risky drugs	84
25	Comparison between cases with low and high abortions as regards to hormonal fixation intake	84
26	Result of Karyotype among studied cases with repeated abortions	85
27	Sex distribution of chromosomal translocation among positive patients	86
28	Comparison between carrier and non carriers as regards history of consanguinity	86
29	Comparison between carrier and non-carrier as regards the mean age of the female and mean age of husbands	87
30	Comparison between cases carriers and non-carriers as regards mean number of abortions:	87
31	Comparison between carriers and non-carriers as regards number of abortion	88
32	Comparison between carriers and non-carriers as regards Environmental Exposure near Residence place	89

## IV

33	Comparison between carriers and non-carriers as regards to smoking of husbands	90
34	Comparison between cases with and without translocations as regards hazardous fertility job of husbands	90
35	Comparison between carriers and non-carriers as regards the mean caffeine intake in males and females	91
36	Comparison between cases with and without translocations as regards indoor exposures to hazardous substances (detergent insecticide and hydrocarbon ) .	92
37	Comparison between cases with and with translocations as regards intake of drugs	93
38	Cytogenetic finding, number of abortions and maternal age in cases with translocations	94

## List of Figures

<b>Figure</b>	<b>Title</b>	<b>Page</b>
1	Morphologically chromosomes are divided into metacentric, submetacentric and acrocentric	14
2	Comparison between cases with high and low abortions as regards maternal and paternal age	72
3	Distribution of high numbers of abortions among studied patients	73
4	The number of abortions according to women's age	75
5	Percentage of abortions related to Men's age	76
6	Frequency of chromosomal abnormality in studied cases	85
7	G-banding karyotyping, translocation carrier male. The patient karyotype was 46,XY,(6,8).	95
8	G-banding karyotyping, translocation carrier Female. The patient karyotype was 46,XX (15,21).	96
9	G-banding karyotyping, translocation carrier Female. The patient karyotype was 45,XX (14,21).	97
10	G-banding karyotyping, translocation carrier Female. The patient karyotype was 45,XX (13,14).	98



## **INTRODUCTION**

Recurrent spontaneous abortion is defined as two or more consecutive pregnancy losses prior to 22 weeks of gestation, usually occurring in approximately the same gestational week. (Rai and Regan 2006).

The World Health Organization (WHO, 1977) has defined spontaneous abortion as the expulsion or extraction from its mother of an embryo or fetus weighing 500g or less, which would normally be at 20-22 complete weeks of gestation

Early spontaneous abortions are defined as those that occur before the 12<sup>th</sup> week of gestation, with late spontaneous abortions being those that occur from 12-20 weeks of pregnancy, and 500g or less (Kallen, 1988; Statistics Canada, 2004).

Spontaneous abortions may be subdivided into unrecognized and recognized spontaneous abortions (Nguyen and Wilcox, 2005) and into first and second trimester spontaneous abortions.

An unrecognized (subclinical) spontaneous abortion occurs after the conception but before the woman is aware she is pregnant, and is detected by measurement of human chorionic gonadotropin (hCG) level in blood or urine.

A recognized (clinical) spontaneous abortion occurs after the woman realizes she is pregnant. The risk of a clinically recognized spontaneous abortion has generally been estimated to 9-15% (Nybo Andersen et al., 2000; Gindler, 2001).

Environmental factors play an important role in the recurrent spontaneous abortion of pregnant mother (Gardella and Hill, 2000).

Several factors involved in human reproduction have been proposed as risk factors including genetic factors (chromosomal abnormalities or balanced translocations of either parent), uterine factors (malformations , trauma , tumors) , endocrine factors (uncontrolled diabetes mellitus, insulin resistance, untreated thyroid disease, hyperprolactinaemia, luteal phase deficiency, hCG deficiency, hypersecretion of luteinizing hormone, polycystic ovary syndrome, premature ovarian failure), immune factors (antiphospholipid syndrome, alloimmune factors) thrombophilic defects (activated protein C- resistance, factor V Leiden mutation, defect protein C or S, defect anti-thrombin III, hyperhomocysteinemia, prothrombin, gene mutation) ,infectious agents (bacterial vaginosis, Chlamydia tracomatis, human papillomaviruses, toxoplasmosis, rubella, cytomegalovirus, herpes, listeria), environmental factors (heavy metals, organic solvents, smoking, caffeine, drugs, hyperthermia, pesticides) ,and psychological factors (trauma, stress).(Cramer and Wise 2000, Gardella and Hill 2000 , Li T.C. et al., 2002 , Dhont 2003, Christiansen et al., 2005, Arredondo and Noble 2006 , Kuttch and Triplett 2006 , Rai and Regan 2006).

## **Aim of the Present Work**

The present study focuses on two main goals:

- Study the effects of genetic factors and environmental factors such as ionizing rays, X-rays, microwaves, ultrasound, and electromagnetic fields), chemical exposure (pesticides, herbicides, aerosol) prescription drugs, air pollution (vapour of cadmium, lead, cigarettes ....etc) drug abuse infectious agents (Cytomegalovirus, toxoplasma....etc) on recurrent spontaneous fetal loss.
- . Association study between the genetic and environmental causes participating in the recurrent spontaneous fetal losses.

## **Human Development**

**H**uman reproduction is a complicated process and the complex transition from a single fertilized ovum to a normally formed human being depends upon a series of precisely timed genetic and environmental interactions (Wilkie et al., 1994).

The development of the human embryo can be divided into three periods:

### **1- Preimplantation period:**

Pregnancy occurs when a sperm penetrates an egg. This is called fertilization and usually takes place in the woman's fallopian tube. This fertilized egg immediately begins to divide into a growing cluster of cells. Between 5-7 days after ovulation the fertilized egg implants into the wall of uterus and starts forming the placenta. The placenta maintains and nourishes the baby by enabling the transfer of O<sub>2</sub>, CO<sub>2</sub>, amino acids, fats, vitamins and minerals from the mother's blood. It also allows transfer of waste substances from the growing baby. During this stage, the zygote undergoes cleavage by a series of mitotic divisions, which gives rise the embryo (Hay, 1998). Errors that occur during this early stage of embryogenesis cause the death of the embryo (Winter and Baraitser, 1998).

### **2- Embryonic period:**

From the time of implantation into the wall of uterus until approximately eighth week of life the baby is known as embryo. Development is rapid during this stage as the specialized cells begin to form the vital organs, nervous system, bones, muscles and blood. During this period cells

differentiate to form different tissues and organs of the embryo. Most major malformations arise during this critical period of organogenesis (Hay, 1998).

### **3- Fetal period:**

After the eighth week of pregnancy the developing baby is called a fetus. It is 2.4 cm. long with most of internal organs formed and external features such as eyes, nose, mouth and ears start to appear. (HON Foundation 2002).

As the fetus and placenta grow and place increasing demand on the mother, phenomenal alternations in metabolism occur. The most obvious physical changes are weight gain and altered body shape. Weight gain is due to increase in breast tissue, blood and water volume in the form of extra vascular and extra cellular fluid. Deposition of fat and protein and increased cellular water are added to maternal stores. The average weight gain during pregnancy is 12.5 kg. During second half of pregnancy plasma lipids increase but triglycerides, cholesterol and Lipoproteins decrease soon after delivery. The ratio of LDL to HDL increases during pregnancy (Moore 1994).

The last stage of development lasts from the third month until birth, during this period few malformations may be arise (Smith and Donnai, 1997).

---

## **Recurrent Abortion**

### **A)Definitions:**

Recurrent pregnancy loss (RPL) is usually defined as two or more spontaneous pregnancy losses (not necessarily consecutive) in the absence of elective medication or surgical measures to terminate the pregnancy (Lee and Siliver 2000).

The term “miscarriage” is synonymous and often used with patient because the word “abortion” is associated with elective termination (Scroggins et al., 2000)

Spontaneous abortion or miscarriage is defined as the involuntary termination of pregnancy before 20 weeks of gestation (dated from the last menstrual period {LMP}) or spontaneous expulsion of fetus below a fetal weight of 500g (Speroff and Fritz 2005).

Miscarriages can be further divided into embryonic losses which occur before the ninth gestational week and into fetal losses which occur at or after the 9<sup>th</sup> to 20<sup>th</sup> weeks of gestation (Lee and Siliver 2000).

Three different groups among RPL patients can be identified and should be assessed separately because the risk of subsequent miscarriage among these groups varies (Daya 2002)

**1- The primary RPL group** consists of women with three or more consecutive miscarriage with no pregnancy progressing beyond 20 weeks gestation.

**2- The secondary RPL group** consists of women who have had 3 or more miscarriages following a pregnancy that progressed beyond 20 weeks gestation, which might have ended in live birth, still birth or neonatal death.

**3- The tertiary RPL group** consists of women who have had at least 3 miscarriages that are not consecutive but are interspersed with pregnancies that have progressed beyond 20 weeks gestation (and might have ended in live birth , still birth or neonatal death) (Christiansen et al., 2005).

Bricker and Farquharson, (2000). Classified pregnancy loss according to the gestational age into Pre-embryonic, embryonic and fetal.

**Tab. (1):** Pregnancy loss classification.

Types of pregnancy loss	Typical gestational (weeks)	Fetal heart activity	Principal diagnostic group	β-hCG level
Preembryonic	< 6	Never	Idiopathic	Low then fall
Embryonic	6 – 8	Never	Oligomenorrhea / idiopathic	Initial rise then fall
Fetal	> 8	Lost	Antiphospholipid syndrome	Rise then static or fall

-Another study confirmed that 70% of missed abortion, defined as an embryo without cardiac activity, had chromosomal abnormalities, most of which represented non-viable genetic defects (Philipp and Kalousek 2002).