SERUM LEVEL OF β-HUMAN CHORIONIC GONADOTROPIN AS A PREDICTOR FOR ABNORMAL KARYOTYPE OF FIRST TRIMESTER ANEMBRYONIC PREGNANCY

Thesis

Submitted for Partial Fulfillment of
Master Degree in Obstetrics and Gynecology

By

AMIR FAWZY YOUSSEF AHMED

M.B., B.Ch., Al Azhar
Mattaria Teaching Hospital

Under Supervision of

Dr. Magdy Mohamed Mahmoud Abd El Gawwad

Professor of Obstetrics and Gynecology
Faculty of Medicine
Ain Shams University

Dr. Ezzat Sayed El-Sobky

Professor of Genetics
Faculty of Medicine
Ain Shams University

Dr. Ibrahim Anwar Ahmed

Assistant Professor of Obstetrics and Gynecology
Faculty of Medicine
Ain Shams University

Faculty of Medicine
Ain Shams University

Acknowledgement

First and foremost thanks to **ALLAH** whose magnificent help was the reason of accomplishing this work

I would like to express my sincere thanks and deepest gratitude to Professor Dr. Magdy Mohamed Mahmoud Abd El Gawwad, *Professor of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, for his gracious supervision, valuable guidance, generous help, support and continuous encouragement through the whole research. I am deeply affected by his noble, character, perfection, care and consideration. I am very much privileged and honored to have his as my supervisor. To him I owe much more than I can express.*

I would like to express my deepest appreciation and gratitude to Dr. Ezzat Sayed El-Sobky, *Professor of Genetics, Faculty of Medicine, Ain Shams University, for giving me the privilege of working under his supervision, patience, constructive criticism valuable guidance, unlimited time and effort.*

I would like to express my deep appreciation and gratitude to Dr. Ibrahim Anwar Ahmed, *Assistant Professor of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, for giving me the privilege of working under his supervision and for his helpful guidance.*

Last but not least I would like to express my deepest thanks and gratitude to my colleagues and to every one in my family.

Dedication

To my Parents. *For their encouragement and support.*

To my ever supporting, loving Wife*, for her tremendous encouragement, support, understanding and love.*

To my lovely son Mahmoud

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ART	Assisted reproductive technology
CGH	Comparative genomic hybridization
CL	Corpus luteum
DR	Detection rate
DS	Down syndrome
FISH	Fluorescent in situ hybridization
GTG	Giemsa-Trypsin-Giemsa
hCG	Human chorionic gonadotrophin
HPL	Human placental lactogen
ICSI	Intra cytoplasmic sperm
IGF	Insulin like growth factor
IL	Interleukin
IVF	In-vitro fertilization
M-Fish	Multiplex fluorescence in situ hybridization
MHC	Major histocompatibility complex
MS	Micro satellite
NT	Nucheal translucency
OR	Odds ratio
PGD	Preimplantation genetic diagnosis
RM	Recurrent miscarriage
RSA	Recurrent spontaneous abortion
TGF	Transforming growth factor
Th	T-helper
TNF	Tumour necrosis factor
URPL	Unexplained recurrent pregnancy loss
LH	Luteinizing hormone
FSH	Follicular stimulating hormone
TSH	Thyroid stimulating hormone
HLA	Human leucocyte antigen
PAPP-A	Pregnancy associated plasma protein A
RCOG	Royal college of obstetrics and gynecology
ACOG	American college of obstetrics and gynecology

INTRODUCTION

Anembryonic pregnancy (blighted ovum) is a frequent form of loss, that is present between ١٦% and ٣٠% of all pregnancies ***(West, ١٩٩٣; Coulam et al., ١٩٩٧)*** and in up to ٤٠% of ectopic pregnancies ***(Emmrich and Kopping, ١٩٨١).***

Early recurrent pregnancy losses still constitute a major dilemma for many obstetricians and infertility specialists. It remains a major area of research in many centers involved in reproduction and immunology ***(Rubio et al., ٢٠٠٥).***

Chromosomal anomalies are present in ٦٠% of all first trimester abortions ***(Coulam et al., ١٩٩٧; Hassold and Hunt, ٢٠٠١).*** Anembryonic pregnancy has been associated with chromosomal anomalies in up to ٦٧% of cases ***(Minelli et al., ١٩٩٣)***; this findings suggests a high probability of gestational loss.

Approximately ٥٠% of such miscarriages are associated with cytogenetic abnormalities, with trisomy being the most frequent, followed by polyploidy and monosomy X ***(Stephenson et al., ٢٠٠٢).***

The earlier the pregnancy loss occurs, the greater the likelihood of genetic causation. Among first trimester

abortions, ٥٠% to ٨٠% show chromosomal abnormalities, usually aneuploidy ***(Simpson, ٢٠٠٧).***

Although routine cytogenetic analysis of miscarriages has remained an uncommon practice to date, the results help the physician to decide whether further investigations are warranted. In addition, the results are useful in the counseling of couples who are trying to understand why their pregnancy ended in miscarriage and to decide whether to try again ***(Stephenson et al., ٢٠٠٢).***

The hormonal changes and maternal adaptations of human pregnancy are considering the most remarkable phenomena in nature. Endocrinologic parameters in early gestation period have been used to predict abnormal pregnancies and identify fetuses that have chromosomal aberrations ***(Engin et al., ٢٠٠٤).***

In the last decade there has been a gradual shift away from the mid trimester screening toward first trimester screening the purported advantages of first trimester screening include early reassurance with normal results, early diagnosis of fetal disorders. So the question was whether these markers were also of value in first trimester screening? ***(Ray et al., ٢٠٠٤).***

AIM OF THE WORK

The purpose is to establish the possible correlation between karytope of first trimester anembryonic pregnancy and serum level of β-hCG.

HUMAN CHORIONIC GONADOTROPIN (hCG)

Chemical Characteristics:

Human chorionic gonadotropin (hCG)

Human chorionic gonadotropin is a glycoprotein, a peptide framework to which carbohydrate side chains are attached. The long half life of hCG is approximately ٢٤ hours as compared with two hours for luiteinizing hormone (LH), a ١٢-fold difference, which is due to mainly to the greater sialic acid content of hCG. As with the other glycoproteins, follicle stimulating hormone (FSH), LH, and thyroid stimulating hormone (TSH) ***(Cunningham et al., ٢٠٠٦)***.

hCG consists of two subunits, called alpha (α) and beta (β). The α subunit in these glycoprotein hormones is identical, consisting of ٩٢ amino acids. Unique biologic activity as well as specificity in immunoassays is attributed to the molecular and carbohydrate differences in the B subunits ***(Speroff et al., ٢٠٠٥)***.

B-hCG is the largest B subunit, containing a larger carbohydrate moiety and ١٤٥ amino acid residues, including a unique carboxyl terminal tail piece of ٢٤ amino acid residues, including a unique carboxyl terminal tail piece of ٢٤ amino acid groups. It is this unique part of the

hCG structure that allows the production of highly specific antibodies and the utilization of highly specific immunologic assays ***(Laphorn et al., ١٩٩٦)***.

The extended sequence in the carboxyl terminal region of B-hCG contains four sites for glycosylation, the reason why hCG is glycosylated to a greater extent than LH, a difference that is responsible for the longer circulating half life for hCG ***(Speroff, ٢٠٠٥).***

All human tissues appear to make hCG, but the placenta is different in having the ability to glycosylate the protein, thus reducing its rate of metabolism and giving it biologic activity through a long half life ***(Gharib et al., ١٩٩٠).***

Biosynthesis

The synthesis of the α and the β chains of bCG is regulated separately. A single gene located on chromosome ٦ encodes the α-subunit of all four glycoprotein hormones – hCG, LH, FSH and TSH. There are seven separate genes on chromosome ١٩ for the β-hCG-β-LH family. Six of these genes code for β-hCG genes are expressed at significant levels. Both the α and β-subunits of hCG are synthesized as larger molecular weight precursors, which are cleaved by microsomal endopeptidases. Once intact h CG is assembled, the molecule is rapidly released from the

cell through exocytosis of secretory granules ***(Miller-Lindholm et al., ١٩٩٧; Cunningham et al., ٢٠٠٦).***

The hCG B subunit promoter does not contain steroid hormone response elements, allowing hCG secretion to escape feedback regulation by the sex steroid, in contrast to FSH and LH. Using recombinant DNA technology, it has been demonstrated that there is a single human gene for the expression of the α-subunit. The gene for the α-subunit shared by FSH, LH, hCG, and TSH is located in chromos $٦p_{٢١-٢٣}$. A single promoter site subjects to multiple signals and hormones regulates transcription of the α gene in both placenta and pituitary ***(Albanese et al., ١٩٩٦).***

The gestational profiles of total amounts (free plus combined) of alpha and beta hCG subunits increased together and peaked at ٩-١٠ weeks of gestation. Thereafter total alpha and beta subunits decreased and subsequently remained stable until term. The decline in total alpha hCG subunit is less marked than that of total beta hCG subunit. The alpha to beta hCG ratio was equimolar until ١٠ weeks of gestation when it increased almost four fold until term ***(Nagy et al., ١٩٩٤).***

The genes that encode for the β-subunits of LH, hCG, and TSH are located in a cluster on chromosome $١٩q_{١٣,٣}$. There are six genes for the B subunit of hCG, and only one for B. LH. Transcription for the six hCG genes, each with different promoter activity, varies and it is not certain why

hCG requires multigenic expression (perhaps this is necessary to reach the extremely high level of production in early pregnancy). It is though that β-hCG evolved relatively recently from B-LH, and the unique amino acid terminal extension of B-hCG arose by a read through mutation of the translation stop codon in the B-LH gene; the DNA sequences of the B-hCG genes and the B-LH gene are ٩٦% identical ***(Maston et al., ٢٠٠٦).***

The genetic complexity for the transcription of B-hCG raises the possibility for mutations of these genes as causes of reproductive problems. A search for B-hCG gene deletions in women with recurrent miscarriage or unexplained infertility and for duplications in women with gestational trophoblastic neoplasia found only normal gene structures ***(Layman et al., ١٩٩٧).***

Cellular Origin

Cellular origin of hCG. The origin of hCG appears to vary depending on the time in gestation. At less than ٥ weeks, hCG expression is observed in both syncytiotrophoblast and cytorophoblast cells. Later in gestation, when maternal serum levels are at their peak, hCG is produced almost solely in the syncytiotrophoblast. The amounts of hCG mRNA for both α- and β-subunits in syncytiotrophoblast from the first trimester are greater than at term, which may be an important consideration when used as a screening procedure to identify abnormal fetuses

(Kurman et al., ١٩٨٤; Beck et al., ١٩٨٦; Maruo et al., ١٩٩٢).

hCG gene expression is present in both cytotrophoblasts, but it is synthesized mainly in the syncytiotrophoblast. The maternal circulating hCG concentration is approximately ١٠٠ IU/L at the time of the expected but missed menses. A maximal level of about ١٠٠.٠٠٠ IU/L in the maternal circulation is reached at ٨-١٠ weeks of gestation ***(Nakajima et al., ١٩٩٠).***

All human tissues appear to make hCG, but the placenta is different in having the ability to glycosylate the protein, thus reducing its rate of metabolism and giving it biologic activity through a long half life. The carbohydrate components of the glycoproteins are composed of fructose, galactose, mannose, galactosamine, glucosamine, and sialic acid. Although the other sugars are necessary for hormonal function, sialic acid is the critical determinant of biologic half life. Removal of sialic acid residues in hCG, FSH and LH leads to very rapid elimination from the circulation ***(Speroff et al., ٢٠٠٥).***

Regulation of hCG Synthesis

hCG production and secretion are the result of complex interactions among the sex steroids, cytokines GnRH, and growth factors. GnRH is synthesized by placental cells; GnRH receptors are present on placental