



# **Study of Placental Growth Factor as a Novel Diagnostic Biomarker for Ectopic Pregnancy**

*Thesis*

Submitted for Partial Fulfillment of M.D. Degree  
in Obstetrics and Gynecology

*By*

**Enas Mohamed Tamer Masoud**

M.B.,B.Ch., Dubai Medical College for Girls (2003)  
M.Sc. Obstetrics and Gynecology – Ain Shams University (2009)

*Supervisors*

**Prof. Essam-Eldin Mohamed Ammar**

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

**Prof. Gamal Farag Mostafa**

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

**Asst. Prof. Tarek Aly Raafat**

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

**Prof. Mona Mohamed Zaki**

Professor of Clinical and Chemical Pathology  
Faculty of Medicine, Ain Shams University

**Faculty of Medicine  
Ain Shams University  
Cairo  
2014**

## ***ACKNOWLEDGMENTS***

*Thanks are all to **ALLAH**, the Compassionate and the Merciful, for helping me finish this work, and for blessing me throughout my life by His Compassion and Generosity.*

*Words fail to express my deepest gratefulness to **Professor Essam-Eldin Mohamed Ammar**, Professor of Obstetrics and Gynaecology, Ain Shams University, for his continuous support, guidance, keen education and his fatherly advice which have been of great help in performing this work,*

*I would like to express my sincerest wishes and deep gratitude for **Professor Gamal Farag Mostafa**, Professor of Obstetrics and Gynecology, Ain Shams University, for his generous efforts throughout this work, his meticulous correction and guidance, his patience and endless support.*

*I would like to express my appreciation to **Dr. Tareq Aly Raafat**, Assistant professor of Obstetrics and Gynecology, Ain Shams University, for his continuous supervision and guidance throughout the work,*

*I am also deeply grateful to **Professor Mona Mohamed Zaki**, Professor of Clinical and Chemical Pathology, Ain Shams University, for her guidance and support throughout the laboratory and technical parts of this work,*

*Thanks fail to express my deepest gratitude and indebtedness to my **Parents**. Because of their continuous help, support and loving advice I have reached this far. To Them, I humbly dedicate this work. Also, I am deeply indebted to my **husband** and my **son** for their great support, encouragement and patience all along. I would like to express my deepest gratitude to my **brothers** and to my whole family, who always support me throughout my life.*

*I will not forget to express my thanks to the staff of Ain Shams University Maternity Hospital and the staff of laboratories of Ain Shams University Hospitals for their help throughout the clinical part of this work. I will not either forget to thank the patients who accepted the participation in this study, and made it possible to complete this work,*

# CONTENTS

	<b>Page</b>
<b>ACKNOWLEDGMENTS.....</b>	ii
<b>CONTENTS.....</b>	iii
<b>LIST OF FIGURES.....</b>	ix
<b>LIST OF TABLES.....</b>	xi
<b>LIST OF ABBREVIATIONS.....</b>	xiv
<b>PROTOCOL.....</b>	xxi
<b>INTRODUCTION.....</b>	1
<b>AIM OF THE WORK.....</b>	11
<b>REVIEW OF LITERATURE.....</b>	12
<b>1. ECTOPIC PREGNANCY .....</b>	12
<b>1.1 Definition of Ectopic Pregnancy .....</b>	12
<b>1.2 History of Ectopic Pregnancy.....</b>	15
<b>1.3 Epidemiology of Ectopic Pregnancy...</b>	18
1.3.1 Increasing Ectopic Pregnancy Rates...	28
1.3.2 Maternal Mortality from Ectopic Pregnancy.....	31
1.3.3 Incidence and Maternal Mortality of Ectopic Pregnancy in Developing Countries.....	36
<b>1.4 Sites of Ectopic Pregnancy.....</b>	46
1.4.1 Tubal Pregnancy .....	48
1.4.2 Abdominal Pregnancy .....	52
1.4.3 Broad Ligament Pregnancy .....	58

1.4.4 Multifetal Ectopic Pregnancy .....	58
1.4.4.1 Heterotropic Ectopic Pregnancy ....	58
1.4.4.2 Multifetal Tubal Pregnancy .....	59
1.4.5 Ovarian Pregnancy .....	60
1.4.6 Cervical Pregnancy .....	62
1.4.7 Cesarean Scar Pregnancy (CSP).....	66
1.4.8 Other Sites of Ectopic Pregnancy.....	69
 <b>1.5 Risk Factors for Ectopic Pregnancy...</b>	 70
1.5.1 Tubal Damage Secondary to Inflammation.....	76
1.5.1.1 Pelvic Inflammatory Disease (PID)..	76
1.5.1.1.1 <i>Chlamydia trachomatis</i> .....	78
1.5.2 Prior Tubal Surgery.....	83
1.5.3 Previous History of Ectopic Pregnancy.....	86
1.5.4 Assisted Reproductive Technologies (ART). ....	88
1.5.5 Contraceptive Use.....	91
1.5.5.1 Contraceptive Devices.....	92
1.5.5.2 Oral Contraceptives.....	94
1.5.6 Developmental Anomalies.....	95
1.5.7 Cigarette Smoking.....	96
1.5.8 Increasing Maternal Age.....	99
1.5.9 Previous Cesarean Delivery.....	100
1.5.10 Other Lifestyle Factors.....	101
 <b>1.6 Pathologic Features of Tubal Pregnancy.....</b>	 104

1.6.1 Gross Findings .....	104
1.6.2 Microscopic Findings .....	105
<b>1.7 Pathogenesis of Ectopic Pregnancy...</b>	<b>107</b>
1.7.1 Embryo-Tubal Transport .....	113
1.7.1.1 Tubal Smooth Muscle Contractility and Ciliary Beat Activity.....	113
1.7.1.2 Nitric Oxide (NO) and Nitric Oxide Synthase (NOS).....	114
1.7.1.3 Interstitial Cells of Cajal (ICC).....	115
1.7.1.4 Prokineticins (PROK).....	116
1.7.1.5 Endocannabinoids (CB).....	118
1.7.2 The Tubal Environment.....	120
1.7.2.1 Sex Steroid Hormone Receptors...	120
1.7.2.2 Uteroglobin.....	125
1.7.2.3 Interleukin-1, Interleukin-8 and Interleukin-6.....	126
1.7.2.4 Leukemia Inhibitory Factor (LIF)..	128
1.7.2.5 Homebox Protein A10 (HOXA10)	130
1.7.2.6 Integrins.....	132
1.7.2.7 Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF).....	133
1.7.2.8 Mucin 1 (MUC1).....	135
1.7.2.9 Trophinin.....	136
1.7.2.10 Activins.....	137
1.7.2.11 The Innate Immune System.....	140
1.7.2.11.1 Intra-Epithelial Lymphocytes.....	140
1.7.2.11.2 Macrophages.....	140

1.7.2.11.3 Natural Anti-Microbial Peptides.	141
<b>1.8 Clinical Presentation of Ectopic Pregnancy</b> .....	143
1.8.1 Symptoms and Signs .....	146
<b>1.9 Biochemical Diagnosis of Ectopic Pregnancy</b> .....	151
1.9.1 Human Chorionic Gonadotropin ( $\beta$ -hCG).....	152
1.9.2 Serum Progesterone.....	162
1.9.3 Novel Serum Biomarkers.....	165
1.9.3.1 Pregnancy-Associated Plasma Protein-A (PAPP-A).....	169
1.9.3.2 Pregnancy-Specific $\beta$ -1-Glycoprotein (Schwangerschaft Protein).....	170
1.9.3.3 Human Placental Lactogen (hPL)..	171
1.9.3.4 Activin A.....	172
1.9.3.5 $\alpha$ -Fetoprotein (AFP) and Cell-Free Fetal DNA.....	174
1.9.3.6 A Disintegrin and Metalloprotease-12 (ADAM-12)....	176
1.9.3.7 Nucleic Acid Markers.....	177
1.9.3.8 Inhibin A.....	178
1.9.3.9 Relaxin and Renin.....	180
1.9.3.10 Creatine Kinase (CK).....	181
1.9.3.11 Myoglobin and Smooth Muscle Heavy-Chain Myosin.....	183

1.9.3.12 Vascular Endothelial Growth Factor (VEGF).....	184
1.9.3.13 Placental like Growth Factor (PlGF).....	189
1.9.3.14 Leukemic Inhibitory Factor (LIF)..	190
1.9.3.15 Glycodelin (Placental Protein-14)..	191
1.9.3.16 Mucin-1 (MUC1).....	192
1.9.3.17 Adrenomedullin.....	193
1.9.3.18 Interleukin-2 Receptors (IL2R), Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Tumor Necrosis Factor (TNF).....	193
1.9.3.19 Cancer Antigen 125 (CA-125).....	194
 <b>2. PLACENTAL GROWTH FACTOR .....</b>	 <b>197</b>
 <b>SUBJECTS AND METHODS.....</b>	 <b>218</b>
• <b>Study Design.....</b>	<b>218</b>
• <b>Study Approval.....</b>	<b>218</b>
• <b>Study Groups.....</b>	<b>219</b>
○ <b>Study Sample Size.....</b>	<b>219</b>
• <b>Inclusion Criteria.....</b>	<b>220</b>
• <b>Exclusion Criteria.....</b>	<b>220</b>
• <b>Subjects' Recruitment.....</b>	<b>220</b>
• <b>Ethics and Patients' Rights.....</b>	<b>221</b>
• <b>Methods.....</b>	<b>221</b>
○ <b>Procedural Steps.....</b>	<b>222</b>
[A] <b>Sample Collection.....</b>	<b>222</b>

[B] Immunoassay Procedure...	223
○ Principle Of The Assay .....	224
○ Reagents.....	224
○ Reagent Preparation.....	225
○ Assay Procedure.....	227
○ Calculation of Results.....	228
● Participants' Data Recording.....	229
● Participants' Follow up.....	230
● Outcome Measures.....	231
● Statistical Methodology.....	231
● Addendum-1 (Informed Consent Forms)	234
● Addendum-2 (Participant's Record Form).....	248
<b>RESULTS.....</b>	<b>251</b>
<b>DISCUSSION.....</b>	<b>263</b>
<b>CONCLUSION AND RECOMMENDATIONS</b>	<b>278</b>
<b>SUMMARY.....</b>	<b>280</b>
<b>REFERENCES.....</b>	<b>285</b>
<b>ARABIC SUMMARY</b>	





## LIST OF FIGURES

<b><u>Figure</u></b>	<b><u>Title</u></b>	<b><u>Page</u></b>
<b><u>No.</u></b>		
1	Case-fatality rate and incidence of ectopic pregnancy for the United States, 1980–1992.....	23
2	Sites of Ectopic Pregnancy .....	47
3	Left ampullary fallopian tube ectopic pregnancy.....	49
4	Cornual ectopic pregnancy .....	51
5	Ruptured ampullary early tubal pregnancy.....	51
6	Early abdominal ectopic pregnancy.....	54
7	Abdominal pregnancy at term.....	56
8	Coronal view of T2-weighted single shot fast spin echo (SSFSE) MRI of an 18 week extrauterine pregnancy .....	57
9	Ovarian ectopic pregnancy .....	61
10	A. Transvaginal sonography, sagittal view of a cervical pregnancy, B. Transverse view of the cervical pregnancy.....	65
11	Transvaginal sonogram sagittal view shows a uterus with a cesarean scar pregnancy (CSP).....	68
12	Hysterectomy specimen with a cesarean scar pregnancy is transversely sectioned at the level of the uterine isthmus and	

	through the gestational sac.....	69
13	Gross Picture of Tubal ectopic pregnancy.....	104
14	Schematic diagram summarizing the potential factors contributing to the development of tubal ectopic pregnancy.....	110
15	Immunohistochemistry for CB1 protein expression in Fallopian tube.....	119
16	The nonpregnant Fallopian tube .....	121
17	The tubal implantation site .....	123
18	Change in the hCG level in intrauterine pregnancy, ectopic pregnancy, and spontaneous abortion .....	159
19	Serum biomarker themes.....	168
20	Diagram illustrating the placenta, amniotic and coelomic cavities, yolk sac and embryo at 7–10 weeks of gestation..	205
21	Hypothesis for the hypoxic- and estrogen-dependent regulation of VEGF/PlGF expression, secretion, and signaling pathways that are possibly involved in the pathophysiology of tubal EP .....	213
22	The stock solution was used to produce a dilution series .....	227
23	ROC curve analysis showing the diagnostic performance of PlGF for discriminating cases from controls.....	262



## LIST OF TABLES

<b><u>Table</u></b> <b><u>No.</u></b>	<b><u>Title</u></b>	<b><u>Page</u></b>
1	Numbers of deaths from ectopic pregnancies and rates per 100 000 estimated ectopic pregnancies; England and Wales 1988–90 and UK: 1991–2008.....	34
2	Joint distribution of causes of maternal deaths.....	35
3	The extrapolated statistics of ectopic pregnancy in the Middle East.....	43
4	Maternal mortality and ectopic pregnancy: main results in studies performed in African developing countries .....	45
5	Some reported risk factors for ectopic pregnancy .....	72
6	Risk factors for ectopic pregnancy .....	73
7	Risk factors for ectopic pregnancy, categorized into high, moderate and low risk factors .....	74
8	Ectopic pregnancy risk scale .....	75
9	Summary of ectopic pregnancy after tubal surgery .....	84
10	Summary of maternally controlled factors; in the development of ectopic pregnancy.....	111

11	Summary of embryo controlled factors; in the development of ectopic pregnancy.....	112
12	Expected change in serum hCG levels in first week of monitoring women at risk for ectopic pregnancy.....	155
13	Comparison between both groups as regard general data .....	252
14	Comparison between both groups as regard type of cases .....	253
15	Comparison between both groups as regard obstetric history.....	254
16	Comparison between both groups as regard general condition and abdominal examination.....	255
17	Comparison between both groups as regard vaginal examination .....	256
18	Comparison between both groups as regard Ultrasound examination.....	256
19	Comparison between both groups as regard PlGF.....	257
20	Distributions of the studied cases as regard pregnancy outcome in the study group.....	257
21	Correlation between PlGF versus other variables among cases.....	258
22	Correlation between PlGF versus other variables among controls.....	259
23	Relation between PlGF versus history of	

---

	ectopic pregnancy among cases.....	259
24	Relation between PlGF versus type of pregnancy termination among cases.....	260
25	Relation between PlGF versus general condition among cases.....	260
26	Validity of PlGF in prediction of ectopic pregnancy.....	261