

TABLE OF CONTENTS

Abstract	i-v
Publication and Presentations	vi
Declaration	vii
Certificate of The Supervisor	viii
Acknowledgements	ix-xi
List of Figures	xix-xxiii
List of Tables	xxiv-xxv
List of Abbreviations	xxvi-xxix
CHAPTER I: INTRODUCTION	
1.1. Introduction	1-14
1.2. References	15-23
CHAPTER II: REVIEW OF LITERATURE	24-45
2.1. Placental Microenvironment	25
2.1.1. Immune cells in decidua	25
2.1.1.1. Extravillous trophoblasts (EVTs)	25
2.1.1.1.1 Human Leukocyte Antigen (HLA) ligands of EVT	26
2.1.1.1.1.1. HLA-G	26
2.1.1.1.1.1.2. HLA-G isoforms	26
2.1.1.1.1.2. HLA-C	27
2.1.1.2. decidual NK (dNK)	28
2.2. Early pregnancy loss	30
2.3 KIR-HLA disbalance in early pregnancy loss	30
2.3.1. 2.3.3. Cytokine and Chemokine environment in early pregnancy loss	31
2.3.2. 2.3.4 Autoantibodies in early pregnancy loss.	32-33
2.4. References	34-45
CHAPTER III	45-71
3.1. Introduction	46-49
3.2. Materials and methods	49-52
3.2.1. Study site, study design and participants	49-50
3.2.2. Blood sample collection and clinical investigations.	50-51

3.2.2.1. Antiphospholipid antibody (APLA): β -2 glycoprotein I (a β 2GP1) IgG/ IgM and anti Cardiolipin (aCL) IgG/ IgM) profile in obstetric participants in relation to pregnancy.	51
3.2.2.2 Antinuclear antibody (ANA) profile in obstetric participants in relation to pregnancy.	52
3.2.3 Statistical analysis	52
3.3. Results	53
3.3.1. Clinical profile of the obstetric participants	53
3.3.2. a β -2 glycoprotein I and anti Cardiolipin antibody profile	54-57
in study cohort and its association with pregnancy failure.	
3.3.3. ANA Ig (GAM) profile in study cohort and its association	58
with pregnancy failure.	
3.4. Discussion	59-60
3.5. References	61-71
CHAPTER Iva	72-125
4a.1. Introduction	72-75
4a.2. Materials and methods	75-84
4a.2.1. Study site, study design and participants	76
4a.2.2. Extraction and Quality Assessment of DNA, RNA, and Protein from studied samples	77
4a.2.2.1 DNA isolation from peripheral blood and umbilical cord blood	77
4a.2.2.2 Isolation of RNA, cDNA and Protein from tissue samples	77
4a.2.3. PCR based KIR genotyping and allotyping of HLA-C	78
4a.2.3.1 HLA-G isoform transcript study	78
4a.2.3.2 KIR genotyping in cohort	79
4a.2.3.3 Copy number variation study of KIR2DL1 and KIR2DS1	81

4a.2.3.4 HLA-C allotyping	81
4a.2.3.5. Transcript expression of <i>KIR 2DL1</i> , <i>KIR2DS1</i> and HLA-G in abortus and placenta.	82
4a.2.4. Expression of KIR2D , HLA-C, HLA-G proteins by ELISA	83
4a.2.5. Validation of KIR2D and HLA -C expression by Immunohistochemistry.	83
4a.2.6. Statistical analysis	84
4a.3. Results	84
4a.3.1 Clinical and demographic profile of the obstetric participants	84
4a.3.2 HLA-G and its association with Early Pregnancy Failure	85
4a.3.2.1 Characterization of HLA-G Isoforms early abortus and placenta tissue.	85
4a.3.2.2. Docking study of HLA-G3, HLA-G4 isoforms	87
4a.3.2.3. Expression of HLA–G in tissue samples	
4a.2.2.4.Validation of expression of HLA-G by Immunohistochemistry	88-89
4a.3.3 KIR-HLA-C study in relation to SAB.	91
4a.3.3.1. KIR-2DL1/2DS1– HLA-C combined genotype in mother neonate pairs	91
4a.3.3.2. KIR Genotype, its expression in conjunction of HLA-C in early abortus tissue	95
4a.3.3.3. Analysis of KIR Genotype and Copy Number Variation in the Study Cohort, and Evaluation of KIR2D-HLA-C Expression	99-100

in Term Placenta	
4a.3.3.3.1 KIR genotype and Copy number variation in study cohort.	101-108
4a.3.3.4.KIR2D-HLAC expression in term placenta	109
4.4. Discussion	111-112
4.5. References	113-125
CHAPTER IVb	126-151
4b.1 Introduction	127-129
4b.2. Materials and methods:	130-135
4b.2.1. Study site, study design, and participants:	130
4b.2.2 Extraction and Quality Assessment of DNA, RNA, and Protein from studied samples	131
4b.2.2.1 DNA isolation from peripheral blood.	131
4b.2.2.2 RNA Isolation and cDNA Preparation	131
4b.2.2.3 Isolation of microRNA and preparation of miR-specific cDNA from placenta.	132
4b.2.3. Association study of HLA-G +3142G/C SNP and 14 bp DEL/IN polymorphism with SAB.	132
4b.2.4. Expression of microRNA levels	132
4b.2.5. Expression of HSF1 and DNMT1 in placenta.	134-135
4b.2.6. Statistical analysis	135
4b.3 Results	135
4b.3.1. Association of HLA-G +3142G/C with SAB	135

4b.3.2. Expression of miRs in placenta.	136-137
4b.3.3. Expression of DNMT1 and HSF1 in placenta.	138-139
4b.4 Discussion:	140-141
4b.5 References	142-151
CHAPTER V	152-196
5.1 Introduction	152
5.2. Material and methods	153-154
5.2.1. Study site, study design and participants	155
5.2.2. Isolation of RNA, cDNA and Protein from tissue samples	156
5.2.2.1. Gene Expression Profiles: IL1B, IL2, IL15, IL18, IL21, TGFB, IFNG, TNF, IL10, VEGFA, Cyclin D, Ki-67, Keratin 18 in Abortus and Placenta.	156
5.2.2.2 Protein expression study of cytokine, chemokines and cell markers.	
5.2.2.2.1 Expression of NK surface marker CD56, growth factor: VEGFA, ki-67, keratin 18 and SOCS 3 proteins by ELISA in abortus and placenta	165
5.2.2.2.2 Cytokine and chemokine study in tissue samples by Cytometric Bead Array in flow cytometry platform.	159
5.2.3. Single cell preparation, phenotyping and NK activation study	159
5.2.3.1 Single cell suspension preparation	
5.2.3.1.1 NK phenotyping and activation status	160-161
5.2.3.2 Gating strategy used	162
5.2.4. Statistical analysis	163
5.3 Results	171

5.3.1. Expression of CD56 protein in tissue samples.	163-164
5.3.2. NK phenotyping and activation status in abortus in relation to early pregnancy loss.	165
5.3.3 Cytokine and Chemokine environment in abortus and placenta.	
5.3.3.1 Cytokine expression study	167-171
5.3.3.2 Chemokine expression study	171
5.3.4 Expression study of growth factor: VEGF-A, cell proliferation and differentiation markers: Keratin 18, and Ki-67 in abortus and placenta	174-177
5.3.5. Expression study cell cycle marker -Cyclin D in abortus and placenta	177-178
5.3.6. SOCs 3 expression in abortus and placenta	178-179
5.4. Discussion	180-183
5.5. References	184-196
CHAPTER VI	196-221
6.1. Introduction	199-201
6.2. Materials and methods	
6.2.1. Study site, study design and participants	201
6.2.2. Staining and expression study.	201-203
6.2.2.1 Tissue fixation and tissue processing	
6.2.2.2 H and E staining	202
6.2.2.3 Protein expression CD56 and VEGFA in abortus by Immunohistochemistry	202
6.2.3. Statistical analysis	202
6.3. Results	202-210
6.3.1. Microscopic Analysis of Histology in Early Abortus and Placenta	202-203

6.3.2 Expression of CD56 and VEGFA by immunohistochemistry	208-209
6.4. Discussion	209-210
6.5. References	211-221
CHAPTER VII	222-228
7.1. Summary	222-225
7.2. Conclusion	226-228
APPENDIX	229-236
Appendix I.	229-232
Appendix II.	233-236