# Role of Intralipid for Women with Idiopathic Recurrent Miscarriage and Raised Levels of Uterine Natural Killer (uNK) Cells in the Endometrium

*Thesis*

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# List of Abbreviations

**aCL** : Anti-cardiolipin.

**ACOG** : American College of Obstetrician & Gynecology.

**Ang** : Angiopoietin.

**APA** : Anti-phospholipid antibodies.

**APC** : Antigen presenting cell.

**APS** : Antiphospholipid syndrome.

**BMI** : Body mass index.

**CXCL-12** : A small cytokine belong to the CXC chemokine family.

**ECM** : Extracellular matrix.

**EVT** : Extra villous trophoblast.

**Fas** : Fatty acids.

**GA** : Gestational age.

**G-CSF** : Granulocyte colony-stimulating factor.

**GDM** : Gestational diabetes mellitus.

**HCG** : Human chorionic gonadotropin.

**HLA** : Human leukocyte antigen.

**HSG** : Hysterosalpingography.

**IL** : Interleukin.

**IVIG** : Intravenous Immunoglobulin.

**IVF** **:** In vitro fertilization.

**IUFD** **:** Intrauterine fetal death.

**KIR** **:** Maternal killer immunoglobulin-like receptor**.**

**LA** **:** Lupus anticoagulant.

**LH** **:** Luteinizing hormone.

**LIT** **:** Paternal lymphocyte vaccinations.

**MMP** **:** Matrix metalloproteinase**.**

**MRI** **:** Magnetic resonance imaging.

**MUFAs** **:** Mono unsaturated fatty acids.

**PCOS** **:** Polycystic ovary syndrome.

**PIH** **:** Pregnancy induced hypertension

**pNK** **:** Peripheral blood natural killer**.**

**PR** **:** Progesterone receptor.

**PUFAs** **:** Poly unsaturated fatty acids.

**RCOG** **:** Royal College of Obstetrician & Gynecology**.**

**RIF** **:** Recurrent implantation failure.

**RPL** **:** Recurrent pregnancy loss.

**SO** **:** Safflower-oil based emulsion.

**sPF** **:** Synthetic pre-implantation factor.

**TGF** **:** Transforming growth factor.

**TIMP** **:** Tissue inhibitor of metalloproteinase.

**TNF** **:** Tumor necrosis factor.

**TPN** **:** Total parenteral nutrition**.**

**uNK** **:** Uterine natural killer.

**uPA** **:** Urokinase plasminogen activator**.**

**VEGFA** **:** Vascular endothelial growth factor A**.**

**VSMCs** **:** Vascular smooth muscle cells.

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# Introduction

Recurrent pregnancy loss (RPL) is defined as the loss of 3 or more consecutive pregnancies and is a stressful condition for both women and clinicians alike. It affects about 1% of all fertile couples trying to conceive ***(Rai et al., 2006, Li TC et al., 2002).*** The incidence of spontaneous pregnancy loss may be much greater than is clinically recognized. Spontaneous pregnancy loss occurs in 12% to 15% of all pregnancies. Thirty percent pregnancies are lost between implantation and sixth week. Maternal age and previous pregnancy losses increases risk of subsequent pregnancy losses ***(Nybo Andersen AM, et al., 2000).*** Despite wide range of investigations, no apparent cause is found in more than 50% of cases and these are categorized as idiopathic recurrent pregnancy loss (***Li TC et al., 2002)***.

The endometrium plays an important role in embryo implantation. Abnormal endometrial function leads to a spectrum of reproductive failure including infertility, pregnancy loss and late pregnancy complications. Currently, 50% of cases of Recurrent reproductive failure Recurrent pregnancy losses (PRL) as well as recurrent implantation failure are of unknown etiology, and an

altered endometrial environment is thought to be associated with this idiopathic condition ***(Toth et al., 2011).***

**Uterine natural killer cells** (uNK) are thought to play a significant role in the establishment and maintenance of early pregnancy. The phenotype of uNK cells differs from the peripheral blood NK (pNK) cells. Ninety percent of pNK cells are $CD56^{dim}$ CD16+ cells and are cytotoxic in nature. In contrast, 90% of the uNK population is of the $CD56^{bright}$ and CD16- phenotype, which has little cytotoxic activity, but are a rich source of many different cytokines and growth factors ***(Vacca P. et al., 2011)***. Although their exact function in implantation remains unknown, several studies have demonstrated significant roles for uNK cells in immunotolerance, trophoblast invasion and angiogenesis ***(Quenby S. et al. 2008, Wallace et al. 2012)***.

The number of CD56+ cells in the endometrium varies through the menstrual cycle; there are few present in the proliferative phase, with numbers increasing in the mid- and late secretory phase of the menstrual cycle and further increasing in early pregnancy ***(Lash et al. 2010)***.

Several studies have reported that uNK cell numbers in the endometrium during the peri-implantation period are elevated in women with idiopathic recurrent pregnancy

loss ***(Tang et al. 2011, Tuckerman. et al.2007)*** and recurrent implantation failure as compared to control subjects.

During the luteal phase, the morphological changes observed in the endometrium occur in a highly predictable pattern. Histological dating of the endometrium has been used for decades to evaluate the histological maturation of the endometrium and to diagnose luteal phase defect. Theoretically, an inadequately developed endometrium can contribute to implantation failure which may manifest as infertility or pregnancy loss. However, the value of routine histological evaluation of the luteal phase endometrium in women with infertility is controversial ***(Coutifaris et al. 2004, Beiyu et al. 2014)***.

Several immuno-histochemical studies from different groups have reported increased numbers of uNK cells in mid-secretory phase endometrium from women with a history of RM ***(Clifford et al. 1999; Quenby et al. 1999; Tuckerman et al. 2007). Quenby et al. (1999)*** noted significantly higher uNK cell numbers in women who went on to a further pregnancy loss compared with those who had a live birth, although a subsequent larger study failed to detect such an association ***(Tuckerman et al. 2007)***. In contrast, by using flow cytometry, there were no detectable altered numbers of endometrial uNK cell numbers in

women with PRL ***(Shimada et al. 2004)***, although Lachapelle et al. **(1996)** did report reduced $CD56^{bright}$ and CD16- and increased CD56 dim CD16+ subsets. Although there are discrepancies in results, immune-histochemical studies have consistently shown increased uNK cell numbers in mid-secretory phase endometrium of women with a well-defined history of PRL.

Immunomodulation therapies such as steroids, intravenous immunoglobulin (IVIG), paternal cell immunization and trophoblast membrane infusion have been proposed with conflicting evidence as to their efficacies ***(Porter et al. 2006).***

Although human maternal and fetal immunology is difficult to investigate, aberrant immune responses and an imbalanced cytokine network may be related to infertility, implantation failures after IVF, and recurrent pregnancy losses ***(Makrigiannakis et. al.2011)***. Women with recurrent implantation failure (RIF) should be tested for inherited and acquired thrombophilias. If thrombophilic tests are normal, women should be tested for immunological causes.

Some findings suggest that increases in the percentage of CD56 (dim) cells and NK cytotoxicity in peripheral blood may be important contributing factors for

both RPL and IVF failure ***(Karami et al. 2012)***. In the past, it had been suspected that IVIG increases implantation rates by reducing natural killer cells number and/or cytotoxicity ***(Kwak et al. 1993; Ruiz et al.1996).*** However, ***Edward et al. (2011)*** observed a beneficial effect of IVIG in women with elevated preconceptional TH1:TH2 ratio and/or %$CD56^{+}$ $CD3^{-}$ **(Ashoush S. et al., 2011; *Edward et al. 2011).***

Human leukocyte antigen (HLA)-DQA1*0505 sharing or the maternal killer immunoglobulin-like receptor (KIR) repertoire is associated with recurrent pregnancy loss (RPL) or repeated implantation failure (RIF) ***(Karami et al. 2012)*** and if abnormal, the patient might then benefit from intravenous immunoglobulin (IVIg) therapy ***(Fatemi et al. 2013)***. IVIg has been successful in the treatment of recurrent pregnancy loss and recurrent implantation failure among women with elevated anti-phospholipid antibodies (APA) and/or NK cell activity ***(Coulam et al. 2012)***. When the pregnancy outcomes of women with a history of reproductive failure and elevated NK cell cytotoxicity treated with intralipid were compared with women treated with IVIg, no differences were seen ***(Coulam et al. 2012)***. Side-by-side comparison showed that synthetic pre-implantation factor (sPIF) is equally effective to inhibit NK cell toxicity at a lower dose than intravenous gamma