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Review Article

Biochemistry Section

Natural Killer Cells: An Insight into its Role in Pregnancy

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ABSTRACT

Tight regulation of immunotolerance at fetomaternal interface facilitates the survival of allogeneic embryo. Natural Killer (NK) cells are immune cells capable of lysing the target cells via the release of cytolytic granules containing perforin, granzyme and granulysin. Besides the peripheral circulation, NK cells are also present in the uterine mucosa. In contrast to peripheral NK (pNK) cells, uterine NK (uNK) cells are not the killer cells, rather they provide a suitable microenvironment in the pregnant uterus making it compatible to the growing foetus and promoting normal placentation, vascular remodeling and trophoblast infiltration, all of which are essential for healthy pregnancy. Although, uNK cells are loaded with cytolytic factors which can lyse the trophoblasts if activated, the control mechanisms mediated by balance between activating and inhibitory receptors block the cytolytic function of these uNK cells. Intensive research has elucidated the roles of NK cells in normal and abnormal pregnancy outcomes, such as recurrent spontaneous abortion. Much of the facts are still to be unraveled to fully understand the underlying mechanisms and to introduce newer and potential treatment modalities to support healthy pregnancy.

Keywords: Cytokines, Gestation, Immunosurveillance, Recurrent pregnancy loss

INTRODUCTION

NK cells that originate from haematopoietic stem cells [1] and account for 10% of blood lymphocytes [2] are the subsets with CD16 and CD56 receptors on their surface [3]. CD16 causes activation of NK cells and mediates Antibody Dependent Cellular Cytotoxicity (ADCC) by binding with Fc region of immunoglobulin (Ig) G while CD56 is a Neural Cell Adhesion Molecule (NCAM) that is involved in homotypic adhesion [4]. Based on the cell expression level of CD56, NK cells can be classified as $CD56^{dim}$ type (account for 90%) with higher CD16 and $CD56^{bright}$ cell type (involves 10%) with lower or absence of CD16 [5]. $CD56^{dim}$ cell types are more cytotoxic while $CD56^{bright}$ cells secrete immunoregulatory cytokines [6]. In bone marrow, NK cells develop from haematopoietic stem cells residing in the $CD34^{+}$ cellular compartment and undergo five phases of maturation namely:

- Phase I: Cells develop as $CD34^{+}CD117^{-}CD94^{-}$ subtype [7]
- Phase II: Cells become responsive to the effect of IL-15 [8], IL-7 [9] and IL-21 [10] which are required for NK cell proliferation.
- Phase III: Loss of $CD34^{+}$ expression [11].
- Phase IV: Differentiation into $CD56^{bright}$ subtype and production of interferon γ [11].
- Phase V: Differentiation into $CD56^{dim}$ subtype and expression of CD16 [12].

All these phases are maintained by stromal cell factor, ligand for tyrosine kinases and cytokines [13].

RECEPTORS AND MODE OF ACTION

The functions of NK cells are regulated by a controlled action between inhibitory and activating receptors. Major activating receptors include Natural Cytotoxic Receptors (NCRs) (NKp30, NKp44, NKp46), NKG2D, NKp80, CD16, CD69 [14] while chief inhibitory receptors are C-type lectin receptors and Killer Immuno globulin-like Receptors (KIRs) [15]. KIRs recognise Human Leukocyte Antigen (HLA)-A, HLA-B and HLA-C while CD94/NKG2 (Natural killer group 2) members recognise HLA-E expressed on target cells [16]. Domination of inhibitory signals by activating signals causes target cell lysis [17].

The cells that lack Major Histocompatibility Complex (MHC)-I protein or express stress induced protein are the target of NK cells [18]. However, sometimes cells with MHC-I are also lysed via activating receptors mediated recognition of ligands induced by stress [19]. Various mechanisms explaining the direct action of NK cells on their targets have been postulated, which are given below:

- Release of cytotoxic granules (containing perforins and granzymes) that cause apoptosis in target cells [20].
- FasL (Fas Ligand) or TRAIL (Tumour necrosis factor related Apoptosis Inducing Ligand) mediated cells lysis [20].
- Secretion of cytokines (like Interferons (IFNs), Interleukin (IL)-2, and IL-12) which inhibit tumour angiogenesis [21].
- Antibody dependent cell mediated cytotoxicity [22].

NK cells can also indirectly act on cellular targets by inducing dendritic cells that enhance cytolytic responses specific to antigens, as they are capable of crossing specific antigens derived from NK cell dependent cell lysis to the T-cells [23].

NK CELLS IN PREGNANCY

Pregnancy, a unique and highly regulated physiological process between two living systems, mother and the foetus, whose success depends on a number of factors like maternal immune tolerance, hormone and cytokine balance, angiogenesis, genetic, epigenetic and environmental effects [24]. Abnormal outcomes of pregnancy are due to the impairment of these regulating factors causing adaptation failure [25]. It was a matter of conundrum for the researches that, how the foetus carrying allogeneic proteins from parental genes escaped maternal immunity and rejection. To understand the underlying reasons several mechanisms that regulated maternal immune recognition and expression of foetal antigens were proposed, the failure of which could result in loss of pregnancy [26].

NK cells, the large granular lymphocytes, are also present in uterine mucosa and are termed as uNK cells. During pregnancy, they populate the uterus densely and have the immunosurveillance role thereby protecting the mother and foetus from pathogens [3].

Though the origin of uNK cells is unclear, different possible sources have been postulated. They might have been developed from utero-resistant $CD34^+$ stem cells [27] or from the immature progenitors that migrated from circulation to the uterine mucosa [28], where their survival is progesterone [29] or IL-15 dependent [30].

uNK cells are the most dominant immune cells in uterine mucosa and account for almost 30% of stromal cells in late secretory phase of endometrium [31]. At the preovulatory phase, only a small number of NK cells are found in endometrium [32] followed by drastic increase in secretory phase of reproductive cycle, due to increase in progesterone level and reaches to a peak if implantation occurs [29], accounting for about 70% of the lymphocytes in the endometrium [33]. Progesterone is required for the survival of uNK cells, thus decrease in its level by the end of reproductive cycle cause uNK cells to undergo death [34].

pNK cell vs uNK cells

pNK cells are $CD56^{dim}CD16^+$ type whereas uNK cells are $CD56^{bright}$ $CD16^-$ type [35]. There is a misconception that uNK cells and pNK cells are same, but it is important to know that these two types of cells are phenotypically as well as functionally different, thus they should be considered as a separate subset of lymphoid cells [36]. Both stimulating and inhibiting receptors mediating NK cell activities are quite different between these two subsets. uNK cells express CD56 and KIRs but are devoid of CD16 or CD57 [37]. The uNK cells are important for trophoblast invasion and vascular remodeling [38] whereas pNK cells are cytotoxic in nature and their increased levels are associated with pregnancy failure [39]. Other striking difference observed is in the level of granularity. Though uNK cell are poorly cytotoxic, but still express much higher amount of cytolytic granules enriched with perforins, granzymes and granulysin in comparison to pNK cells [14].

pNK in Pregnancy

Hormones regulating pNK cells activities include prolactin, estrogen and progesterone [24]. The pNK cells show decreased lytic function [40] with the increase of inhibitory receptors expression during the first week of gestation, followed by reaching to maximum level till the third month and to basal level at the end of gestation [41]. In vitro and in vivo animal studies have shown inhibitory effect of estrogens and progesterone on NK cell cytotoxicity either by suppression of NK output from bone marrow or by suppressing NK cellular cytotoxicity individually [24]. Prolactin, at normal concentration, induces proliferation of pNK and its response to IL-2 but it suppresses the response of pNK to IL-2 at higher levels [42].

uNK CELLS IN PREGNANCY

In pregnancy uNK cells concentrate at the site where placenta lies in close vicinity to the infiltrating trophoblasts [43] particularly around spiral arteries and basement membrane [44], thus regulating maternal immune response against foetal allograft and trophoblast growth [24]. The uNK cells also regulates placentation [45], pattern and depth of trophoblast invasion [46].

Though the exact function of uNK cells is not known completely, their location indicates them to be involved in foetal implantation [31], vascular remodelling and trophoblast invasion [47]. The uNK cells secrete growth factors required for switching the endometrium from menstruation to decidualisation during pregnancy [48]. Recently, NK cells receptors for trophoblast MHC-I proteins have been discovered which has opened a new door for the study of uNK cells function [36]. However, in absence of pregnancy, uNK cells undergo apoptosis [24] playing an important role in breakdown of endometrium and causing menstruation [49].

Vascular Remodeling and Angiogenesis

After implantation, it is essential to ensure sufficient supply of nutrients and oxygen to growing foetus [46]. The uNK cells along with trophoblasts [50] promote transformation of endometrial vessels and spiral artery into wide and low resistance canals allowing adequate supply of blood and nutrients from mother to foetus [25]. Initially, there is loosening of media [51], which after implantation is destroyed and replaced by Endovascular Trophoblasts (EVTs) [52]. During this process several angiogenic factors are secreted by uNK cells that affected vascular stability as well as function [53]. Soares MJ et al., demonstrated hypoxia and delay in spiral artery transformation in uNK cells depletion [54]. Major angiogenic factors produced are Vascular Endothelial Growth Factor C (VEFG-C), IL-8, placental growth factors, IP10 and angiopoietins [55]. These factors also maintain non-cytotoxic phenotype of uNK cells [56].

Trophoblast Invasion

It is a complex process accompanied by a series of changes in uterine mucosa, collectively known as decidualisation, which includes glandular and stromal element differentiation, increase in spiral artery tortuosity etc [57]. Trophoblast, the foetal cells directly in contact with uterus, are trophectoderm derived cells surrounding blastocysts providing shelter to the foetus inside the cocoon [58]. For normal pregnancy, maternal decidua must be invaded physiologically by trophoblasts so as to promote the adequate oxygen supply for the development of feto-placental unit [59]. Trophoblast invasion is regulated by a number of factors secreted by uNK cells. In vitro studies have reported that IL-15 stimulates the process while TNF-α and INF-γ have the inhibitory roles [60].

The uNK cells via its wide range of receptors can respond to signals which are either induced due to hormonal changes during pregnancy or expressed by trophoblasts [46]. The intimate contact of maternal immune system with trophoblasts causes encroachment of epithelial boundaries by allogeneic cells [46]. Cytotrophoblast in villi, that is in contact with maternal circulation in intervillous space lack HLA-A, HLA-B, HLA-DP, HLA-DQ and HLA-DR [3] but the deeply infiltrating EVTs express characteristic MHC proteins e.g, HLA-E, HLA-G [61] and HLA-C [62], that transits signals to immune cell of mother, causing either activation of inhibition of uNK cells depending upon the functional role of maternal KIRs. The number of uNK cells that express KIRs specific to HLA-C1 and HLA-C2 increases in early pregnancy [43].

Depending upon the interaction between HLA-C and KIR, the amount of cytokine produced by uNK cells vary, that in turn affect the trophoblast invasion [63]. Inappropriate trophoblast invasion is one of the major causes for early pregnancy loss [64].

Cytokine Production

According to earlier reports, NK cells are capable of producing Type 1 and Type 2 (major) cytokines [65]. Type 1 category mediated cellular immunity and thus was regarded detrimental for maintaining normal pregnancy while Type 2 evoked humoral immunity which was shown to have a protective role for the foetus [66], suggesting the dominance of Type 2 cytokines over Type 1 during pregnancy [66]. Examples of Type 1 cytokines include IL-2, IL-12, INF-γ, and TNF-β whereas those of Type 2 cytokines are IL-4, IL-5, IL-6, IL 10 and IL-13 [67]. These cause migration of cytotrophoblasts to decidua basalis via interactions with CXC Chemokine Receptor (CXCR) 1 and 2 receptors on trophoblasts [63] thereby favouring vascular remodelling required for placental development and normal pregnancy [68].

Immunomodulation

Foetal protection from maternal immune system is essential for successful pregnancy outcome [69]. Gene expression analysis in human uNK cells have shown overexpression of immunomodulators such as Glycodelin A, Galectin-1 [70] and Tim-3 [71]. Glycodelin A, also called placental protein 14 or progesterone associated endometrial protein [72], causes inhibition of T-cell activation [73]. Glycodelin appears after Luteinizing Hormone (LH) surge but is absent in proliferative endometrium [74]. It is selectively expressed in uNK cells and is involved in local immunosuppression at fetomaternal interface [70]. Galectin-1 suppresses T-cell proliferation and survival, decreases TNF-α, IFN-γ and IL-2 production from T-cell and IL-12 from infected macrophages [75]. Uterine hormones also mediate the action of uNK cells by acting on intermediary cells like T lymphocytes (promotes Th2 environment in uterus) and stromal cells of endometrium [76]. The uNK cells also express high amount of Tim 3 which is further increased in activation [77]. According to Gleason MK et al., Tim-3 acts as a coreceptor and promotes production of INF-γ by NK cells [78]. In a study of Sun J et al., it was reported that Tim-3 suppresses cytotoxicity of NK cells via Galectin-9 dependent pathway. As degranulation is the preliminary requirement of NK cells toxicity, Tim-3 causes suppression of NK cells degranulation and thus reduced level of Tim-3 can be linked with abnormal pregnancy or may be regarded as marker of abortion risks in early pregnancy [79]. Li YH et al., reported Tim-3 positive uNK cells in human miscarriages [80] while Miko E et al., demonstrated decreased Tim-3 in the patients with pre-eclampsia [71].

ANTIGENIC CROSS LINK BETWEEN FETOMATERNAL SYSTEM/ CYTOLYTIC ACTION OF uNK CELLS

Foetal tissue acts as a dynamic organ with the capability of presenting antigens, producing immunomodulators, expressing toll-like receptors that detect internal and external signals to activate maternal immunity and secretion of an array of proteases [81]. The foetus is targeted by maternal uNK cells only if the maternal immune system recognises it as a foreign body [44]. Generally, uNK cells are in contact with trophoblasts of the placenta only but not with embryo. The pNK cells target virus infected cells or tumours by release of cytotoxic granules (perforins, granzymes and granulysin) forming the synapse between the killer cell and the target cells [41]. In contrast to pNK, uNK cells though possessing complete killing machinery for destroying the trophoblast, show non-cytotoxic cross link with the target [82].

CD69, which acts as an earlier marker of NK cells activation [83], not only mediates NK cell dependent cytotoxicity but also causes NK cell proliferation, TNF-α production and expression of activation antigens [84]. In vitro studies have shown that, CD69 activated NK cells can lyse trophoblasts, thereby increasing the risk of miscarriage and failure of in vitro fertilisation procedure [85]. Studies conducted in vitro in mouse demonstrated extravillous cytotrophoblast apoptosis induced by release of granulysin from the uNK cells, thereby causing spontaneous abortion [86].

Study of Thum MY et al., revealed that women with In Vitro Fertilization (IVF) failure have significantly high levels of CD69 NK cells in blood which suggests that $CD69^{+}$ NK cells have negative effects on successful implantation [39].

Human body consists of control mechanisms to block the cytotoxic functions of uNK cells. As indicated by a number of studies, the balance between activating and inhibitory NK cells receptors is the major contributing factor [87]. In addition to KIRs, three other inhibitory receptors specific to uNK cells have been reported viz ZB4 (anti fas antibody receptor), LILRB1/ILT2 (Leucocyte immunoglobulin like receptor subfamily 1/ Immunoglobulin like transcript 2) and CD94/NKG2A. ZB4 is expressed by all uNK cells whereas rest are expressed only by a subset of uNK cells [14]. Specific interaction of inhibitory uNK receptors with MHC-Ia or MHC-Ib molecule [88] drastically inhibited cytotoxicity of uNK cells in decidua basalis [89]. Interaction of LILRBI/ILT2 receptors with HLA-G also inhibits uNK cellular cytotoxicity [90]. It is also documented that release of VEGF-C by uNK cells in early pregnancy causes up-regulation of TAP-1 in cytotrophoblasts, thus protecting them from lysis [56].

EFFECT OF uNK CELL ON PATHOGEN INFECTED DECIDUAL CELL

Decidua can be infected by several pathogens like human *cytomegalovirus* (hcMV) [91], HIV-1 [92], *Toxoplasma* [93], *Plasmodium falciparum* [94], hepatitis virus [92] etc., resulting in spread of infection to foetus from the mother. However, uNK cells provide local immunity protecting the foetus from infection via secretion of local cytokines or chemokines [95].

NK CELLS IN RECURRENT PREGNANCY LOSS (RPL)

Miscarriage occurring for three times consecutively is called RPL and affects about 1%-3% of pregnancies [96]. The uNK cells maintain highly regulated balance between trophoblast invasion and abnormal placentation, as the latter is known to increase the risk of RPL, implantation failure, preterm birth, pre-eclampsia, preterm premature rupture of membrane etc., [97] which are collectively known as Great Obstetrical Syndromes [98].

Role of pNK

For normal and successful pregnancy, there should be T helper cell 1/ T helper cell 2 (Th1)/(Th2) balance. Increased Th1 activity has been linked with RPL while increased Th2 activity is favourable for the progression of pregnancy. Increased pNK cells are associated with increased Th1 activity and movement of cytotoxic cells to uterus resulting in pregnancy loss. On the other hand, decrease in pNK cells is linked with enhanced Th2 activity and decreased cytotoxicity in uterus thereby favouring the progression of pregnancy [99]. However, the mechanism lying behind still remains to be established.

Ntrivalas El etal., showed higher expression of CD69 and decreased expression of CD94/NKG2 inhibitory receptors on NK cell of women with RPL [100]. This suggests that imbalance in expression of CD69 and CD94 in pNK cells in RPL cases may be responsible for their pathology. Besides this, pathogenic changes attributed to altered hormonal regulation of NK cells also affect the pathophysiology and requires additional investigation.

Role of uNK

According to Kitaya K et al., [101], in women with RPL, there is increase in the number of $CD56^{dim}$ $CD16^{+}$ subtype while $CD56^{bright}$ $CD16^{-}$ subtype decreases. As CD16 causes ADCC, the NK cells positive for $CD16^{+}$ show greater lytic activity than $CD16^{-}$ cells, indicating the increased number of $CD16^{+}$ NK cells in women with RPL to be the plausible reason for pregnancy loss. Further, $CD56^{bright}CD16^{-}$ NK cells produce hormones essential for placental growth and a decrease of which can develop an unfavourable environment for the growing foetus causing pregnancy failure [23]. However, further larger studies are required to support these findings. A screening of role of uNK cells on RPL has indicated several possible observations as follows:

- uNK cell responsible for pathophysiology of RPL may be different than uNK required for successful pregnancy, which is attributed to Th1/Th2 cytokine imbalance.
- uNK cells that are normally present on endometrium may not be involved in pathogenesis of RPL, rather pNK cells which are normally absent in endometrium could be the real culprits.
- Defective production of IL-4 and Lipopolysaccharide (LPS) by decidual T-cells.

- Decreased production of glycodelin that results in uterine flushing [24].

uNK cells testing and therapeutic approach in RPL

The uNK cells can be tested by endometrial biopsy but drawback is that it can be tested in the pregnancy period only. In most of the studies uNK cells are analyzed immunohistochemically. Though this method is more appropriate to locate the NK cells, it is more time consuming in comparison to flow cytometry. In flow cytometry, there is requirement of large volume endometrial samples due to higher chances of cellular and antigen loss caused by tissue digestion and the process may be difficult in some women from whom such large volumes of samples cannot be obtained [102]. Various therapeutic regimens for RPL has been developed including heparin, human chorionic Gonadotropin (hcG), progesterone, prednisone, aspirin, Intravenous Immunoglobulin (Iv Ig) and leukocyte immunization. Interestingly only heparin and progesterone have been shown to significantly affect the NK cells function. Heparin suppressed cytotoxicity of NK cells and inhibited binding of INF-γ to cell surface [24] while progesterone inhibited release of cytokines from TH cells and decreased the embryo toxicity [103]. However use of heparin as well as aspirin is controversial in thrombophilia and requires further evidences.

Farghali MM et al., recently used conventional G-banding techniques to analyze chromosome of the monolayer cell culture obtained from abortous specimen. In this process immunohistochemical staining was carried out with monoclonal antibody specific to CD56, CD16 uNK cells [104]. Another therapeutic approach that has been developed recently is the use of Synthetic Peri-Implantation Factor (SPIF). PIF is secreted by embryo and is required for the implantation and the trophoblast invasion. Roussev RG et al., in their study involving 107 women with RPL and 26 women with IVF, used SPIFs as drugs for treatment. They found that SPIFs decreased NK cells toxicity by suppressing the expression of NKCD69 suggesting it to be effective, safe and non toxic therapy for RPL [105].

Recently, trials have been carried out to correlate uNK cell numbers with successful implantation during the luteal phase [106]. But the major problem encountered was the large variability of uNK cells during menstruation cycle, followed by rapid surge after ovulation, which was mediated by progesterone induced activation of IL-15 in the stromal cells [107]. Therefore any alteration in hypothalamic-pituitary-ovarian axis will produce changes in uterine mucosa along with NK cell proliferation [108].

A summary of different studies involving analysis of pNK and uNK

S. No.	Study	Number of subjects	Analysis technique	Cells	Inclusion criteria	Outcome
1	Yamada H et al., (2003)	113	Flow cytome try, Cr release assay	pNK	Patients with ≥ 2 miscarr iages	Higher percentage of NK cells in females with miscarriages [109].
2	Thum MY et al., (2007)	138	Flow cytometry	pNK	Patients subject ed to IVF	No difference was observed in NK cells and its subset percentage among infertile females and those who achieved pregnancy after Assisted Reproduction Technique (ART) [110].
3	Matsubayashi H et al., (2005)	94	Cr release assay	pNK	Patients subjected to IVF	NK levels were high in infertile women who failed to achieve pregnancy and achieved pregnancy after ART [111].
4	Baczkowski T and Kurzawa R (2007)	58	Flow cytometry	pNK	Patients subjected to IVF	Difference could not be obtained with regard to NK cell percentage among infertile females and those who achieved pregnancy after ART [112].
5	Katano K et al., (2013)	552	Endom etrial biopsy	pNK	Patients with 2-6 miscarr iages	pNK cells are independent risk factors for miscarriages. Thus, pNK activity should not be measured in RPL as its clinical significance is still to be elucidated [113].
6	Ledee Bataille N et al., (2004)	15	Immuno histo chemistry	uNK	More than 3 IVF failures	No difference in NK cell count among infertile women who failed to achieve pregnancy and those who achieved pregnancy after ART [114].
7	Liu B et al., (2014)	84 (RPL) 72 (IVF Failure)	Histolog ical dating	uNK	RPL	Less developed endometrium in women with miscarriages and uNK cell count in combination with histological dating has higher prognostic value [115].
8	Tuckermen E et al., (2007)	87	Immnun ohisto chemistry	uNK	Patients with 2-3 miscarri ages	Significant difference in NK cell could not be obtained in females with miscarriages and those who achieved pregnancy after ART [116].
9	Seshadri S and Sunkara SK (2014)	review of 22 meta-analysis study	Search of electronic databases like medline, embase etc. Review of known primary articles to identify articles not captured in databases.	pNK and uNK	RPL	No significant difference in uNK cell and pNK cells among fertile and infertile females when percentage rather than absolute count is considered Live birth rate was not significantly different in patients with high or low levels of NK cells. Further researches required to recommend NK cell analysis as diagnostic tool [117].

[Table/Fig-1]: Studies analysing pNK and uNK cells.

S. No.	Study	Type of study	Number of sub-jects	Therapeutic approach	Outcome
1	Ata B et al., (2011)	RCT (randomized control trials)	160	Predni sone	No complications or adverse foetal outcomes [118].
2	Tang AW et al., (2013)	Review	272	Intravenous immunoglo bulin	Treatment did not offer benefit to the patients with RPL. As per the authors, unless underlying mechanisms and patient who might gain benefit from iv Ig therapy are not identified, iv Ig for RPL is not recommended [119].
3	Wong LF et al., (2014)	Review	303	Intravenous immunoglo bulin	No significant difference among control and patient in terms of live births [120].
4	Roussev RG et al., (2013)	RCT	107	SPIFs	SPIFs decreased cytotoxicity of uNK cells in females with RPL by suppressing expression of NKCD69 [105].
5	Moffett A and Colucci F (2014)	Review	-	Various different therapies	Authors concluded that use of various therapies for RPL is due to misconception and can lead to serious side effects. So it is not feasible to use such therapies to alter uNK cell activity until known more from larger studies [46].

[Table/Fig-2]: Studies analysing various therapeutic approaches for RPL.

cells with the therapeutic approach to RPL and their controversial outcomes are given in [Table/Fig-1,2].

However, all the therapeutic modalities that has been developed lack reliability and their use as potent therapeutic regimen for females with RPL requires evidences from further large scale studies, therefore more studies in pNK cells and uNK cells involving appropriate inclusion criteria, methodology and test result are needed. Unless clinically sound evaluation and therapeutic modalities are obtained from larger studies, women with reproductive problems must not be subjected to NK cells analysis or immunotherapy as a routine practice. Rather they can be counseled about lack of evidences and encouraged to be part of studies designed to rule out NK cell as a clinical marker for screening so that, it can be safely and effectively used in near future.

CONCLUSION

Although, uNK cells have cytolytic potential, they show beneficial role in pregnancy by facilitating placental development, angiogenesis and trophoblast invasion. Assuming pNK cells to be similar to uNK cells, it has become a common practice to examine pNK cells in women with infertility and RPL. However, uNK cells and pNK cells being quite different from each other, analysis of pNK cells can rarely give any significant picture of uterine abnormalities. In vitro cytotoxic assays for NK cells may not be relevant to its in vivo functions. Therefore, clinically significant information cannot be gained. It is also unclear whether uNK cells affect earlier implantation stage when blastocysts penetrate the endometrium. There are still some gaps which need to be filled via larger scale studies or genetic studies before researches on uNK cells could be translated to therapies. Though several studies have been shown involvement of uNK cells in successful pregnancy outcomes, the exact underlying mechanism is still a question. This should be elucidated by further investigations, so that it could be helpful in identification of new targets and development of effective therapies for healthy pregnancy.

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