The role of uterine natural killer cells in recurrent pregnancy loss and possible treatment options

Yentl Louise Betty Nel Béquet, M.D.,[a] Eileen Elisabeth Lynn O'neill Lashley, M.D., M.Sc., Ph.D.,[a] Mariette Goddijn, M.D., Ph.D.,[b] and Marie-Louise Petronella van der Hoorn, M.D., M.Sc., Ph.D.[a]

[a] Department of Gynecology and Obstetrics, Leiden University Medical Center, Leiden, the Netherlands; and [b] Department of Gynecology and Obstetrics, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam University Medical Center Location AMC, Amsterdam, the Netherlands

This narrative review summarizes the current knowledge on the role of uterine natural killer (uNK) cells in recurrent pregnancy loss and possible treatment options. Recurrent pregnancy loss involves 2 or more consecutive miscarriages, affecting around 3% of couples attempting conception. Despite extensive investigation, causes often remain elusive. Uterine natural killer cells, critical in early gestation and implantation, may hold answers for treatment options. Properly designed and powered clinical trials are needed to provide more answers on the effect of treatment options in relation to uNK cells. (Fertil Steril® 2023;120:945–7. ©2023 by American Society for Reproductive Medicine.)

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A common complication in pregnancy is miscarriage, defined as the spontaneous demise of a pregnancy before the fetus reaches viability. Recurrent miscarriage, also known as recurrent pregnancy loss (RPL), is internationally referred to as 2 or more consecutive miscarriages and affects around 3% of couples trying to conceive (1, 2). In the absence of embryonal chromosome abnormalities, RPL can be attributed to lifestyle, autoimmune, endocrine, anatomic, or parental genetic related factors. Despite extensive diagnostic investigations, the causes of RPL often remain unexplained (3). Because the fetus is a semi-allograft, which escapes maternal immune rejection in healthy pregnancy, possibly the immune system plays a role in the mechanism of such unexplained RPL cases. The uterine natural killer (uNK) cells is the most prominent during early implantation, accounting for more than 70% of all leukocytes (4). Here we highlight the current understanding of uNK cells, including their function and how these cells can be possibly targeted within the context of treatment options for RPL.

uNK CELLS

Our understanding on the role and the characteristics of uNK cells has increased significantly over the last years. Natural killer (NK) cells are part of the innate immune system and are present in the peripheral blood and can also be found in the uterus, more specifically the endometrium, as well as in other lymphoid and non-lymphoid tissues (5). These NK cells are large granular cells and are characterized by their ability to both lyse cells and secrete cytokines. Within the NK cell population, 2 primary subsets can be identified: CD56dimCD16+ cells, which constitute the majority (>90%) of the cells in peripheral blood, and the other main subset (10%), CD56brightCD16-; the uterine NK cells (4). Uterine natural killer cells have a phenotype that differs from most peripheral blood NK cells. They express high levels of the NK cell marker CD56 while lacking the CD16 marker. Consequently, their role is primarily immunological response regulation through cytokine production rather than cell lysis through their cytotoxic potential (6). The population of uNK cells in the endometrium fluctuates throughout the menstrual cycle and can be influenced by hormonal factors, such as the use of progesterone-based contraceptives (4). Uterine NK cells are typically most abundant during the late secretory phase of the menstrual cycle and in the first trimester of pregnancy. After this period, uNK cell numbers diminish until <3% at term (4, 5). Possibly, uNK cells play a role in implantation by secreting cytokines and angiogenic factors, such as transforming growth factor-β, angiopoietin 1 and 2, vascular endothelial growth factor, and placenta growth factor (6). In endometrial samples, uNK cells surround the spiral arteries, indicating their involvement in facilitating proper trophoblast invasion, vascularization,

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Correspondence: Yentl Louise Betty Nel Béquet, M.D., Department of Gynecology and Obstetrics, Leiden University Medical Center, Leiden, the Netherlands (E-mail: y.l.b.n.bequet@lumc.nl).

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and placentation (5). However, the exact function of uNK cells is currently not completely understood.

uNK CELLS AND PREGNANCY LOSS

It is theorized that an excessive number of uNK cells or overly cytotoxic uNK cell population could lead to increased peri-implantation blood flow and excessive oxidative stress to trophoblast cells, thereby increasing the risk of pregnancy loss (7). Indeed, super-physiological number of CD56+ uNK cells in endometrium seems associated with unexplained recurrent pregnancy loss (5). However, no differences were detected in a meta-analysis pooling all results on CD56+ CD16- cells in endometrium of RPL patients than in control patients (7). Moreover, studies examining the relationship between the level of uNK cells and the prediction of successful subsequent pregnancies in women with RPL have shown contradictory results over the years (8–10). The above-mentioned studies have certain limitations, including small sample sizes and low methodological quality. In addition, there is heterogeneity among the studies because of the use of varying definitions of RPL, control group variations, different analysis methods, and distinct cut-off values (7). Also, previous studies have measured uNK cell levels in pre-pregnancy endometrial samples using invasive techniques such as biopsies or curettes (8–12). The timing of sampling is crucial because of uNK cell fluctuation during the menstrual cycle, which has been overlooked in previous studies. Moreover, uNK cells are unevenly distributed in the endometrium, affecting cell density measurements. Interestingly, van der Molen et al. (13) proposed an alternative and user-friendly method using a menstrual cup. Their study demonstrated comparable, highly reliable, and highly reproducible results, suggesting the potential for wider adoption of this method for analyzing endometrium derived immune cells in future research (13).

In addition to levels of uNK cells, also the function of uNK cells has been extensively studied in literature. There is a wide repertoire of uNK activity that can be measured, including recruitment and regulation mechanisms and production of cytokines, cytotoxicity, and angiogenesis. Studies on uNK cell activity have also shown conflicting findings because of above-mentioned methodological shortcomings (7).

The interactions between uNK cells and other cells in the uterine environment are intricate, influenced by constantly changing hormone levels. Consequently, despite the vast literature on this topic, the role of uNK cells in early pregnancy, particularly in early pregnancy complications, remains speculative at present.

uNK CELLS AND PREDNISOLONE

Despite the lack of a well-known pathophysiological mechanism, several therapeutical interventions targeting the maternal immune system to influence pregnancy outcome have been studied. The expression of glucocorticoid receptors on uNK cells and the potential correlation between an increased level or cytotoxic uNK cell population and RPL, suggest a possible approach of using corticosteroid treatment to modulate the immune response (5). Glucocorticoids could potentially improve the intrauterine environment by reducing the uNK cell count, normalization of the cytokine expression profile in the endometrium, suppression of endometrial inflammation, and induction of Tregs. In women with RPL, administration of prednisolone (20 mg daily from day 1 to day 21 of the menstrual cycle) led to a significant reduction in uNK cell numbers from a median of 14% before treatment to 9% after treatment (14).

The evidence to support administration of prednisolone to women with RPL to increase the live birth rate is, however, not convincing at this moment. Despite the lack of evidence, prescription of prednisolone to prevent future pregnancy loss is not rare. A recent feasibility trial involving endometrial biopsies in women with RPL and high uNK cell density (>5%) was conducted. Forty pregnant women in this trial were randomized between placebo and prednisolone in early pregnancy. Although the aim of the study was the feasibility of this approach and not to investigate effectiveness, still, a trend for higher live birth rate in the prednisolone group than in placebo group was found (RR 1.5; 95% CI 0.79–2.86). The effect of prednisolone on the level of uNK cells in decidua was not evaluated in this trial (15).

Another randomized controlled trial including 160 women with unexplained RPL, showed an increased rate of ongoing pregnancy beyond 20 weeks in the group of 74 women receiving prednisolone (5 mg/day) treatment than in 76 women receiving placebo (RR 7.63; 95% CI 3.70–15.70). There was, however, no difference between the initial serum levels of NK cells markers CD56 and CD16 at 20 weeks gestation. The study was also underpowered and both the intervention as placebo group received co-treatment with low dose aspirin and heparin. It is therefore difficult to attribute the effect completely to prednisolone (16). Surprisingly, the study showed an extreme low live birth rate in the placebo group of 9.2%, whereas this percentage ranged between 63% and 65% in recent randomized controlled trials conducted in unexplained RPL group (17, 18).

Finally, a single-blinded randomized controlled trial was performed, enrolling 170 patients with RPL who were assigned to one of 3 groups: enoxaparin until birth; prednisolone, progesterone, and aspirin during pregnancy; or placebo. The results of this study showed a significantly higher live birth rate in both the enoxaparin; and combination therapy group than in the placebo group. Also in this study, the presence of co-interventions involving heparin and aspirin further complicates the assessment of prednisolone's independent impact on the outcomes (19). Remarkably, the trial was powered on a difference in live birth rate of 45%, a difference that is impossible to reach based on the live birth rate in placebo groups of earlier trials. This highlights that there is a need for higher quality studies on the effect of prednisolone in RPL.

Because 90% of prednisolone is inactivated by placental metabolism, treatment in low dosage during first trimester seems safe and is a potential useful therapy in women with unexplained recurrent miscarriages. However, it is crucial to consider the possible risks and consequences associated with administering glucocorticoids to women who have experienced RPL (20) and therefore clinical trials with adequate

randomization and power are desperately needed. This was also recommended by the European Society of Human Reproduction and Embryology in their recent guideline on the management of RPL (1).

In conclusion, the lack of certainty regarding the correlation between uNK cell levels, cytotoxicity, RPL, and potential treatments such as corticosteroids can be attributed to several factors. First, there are variations in the definition of RPL and variations in the control groups leading to inconsistencies. Second, the normal range of uNK and peripheral NK cell levels is not well established, nor is the correlation between uNK cells and those in peripheral blood. In addition, confounding factors, such as the use of co-medication in interventional studies, further trouble the interpretation of results. Finally, the limited sample sizes in conducted research studies contribute to the challenge of comparing data and drawing definitive conclusions (4).

FUTURE PERSPECTIVES

Recurrent pregnancy loss is associated with increased obstetric complications, physical and psychological burden, and significant healthcare costs (3). New well-designed interventional studies with significantly large sample sizes could improve the care for couples with RPL and therefore it is crucial to prioritize these studies. Currently, there are 4 registered randomized controlled trials in the International Standard Randomized Controlled Trial Number and clinicaltrials.gov databases. These trials aim to investigate the impact of corticosteroids on uNK cell levels and/or pregnancy outcomes in individuals with RPL. Hopefully, the results of these future studies, preferably combined with an individual patient data meta-analysis in the near future, will provide clear and promising insights on the effect of corticosteroid treatment in women with RPL. Together with biological tissue examination, these studies may help determine whether uNK cells play a role in predicting the treatment's efficacy.

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