IMPACTO CIENTÍFICO EDITORA

UTERINE AND PERIPHERAL NK CELLS IN RECURRENT SPONTANEOUS ABORTION: A QUALITATIVE SYSTEMATIC REVIEW OF BIOMARKER MECHANISMS (KIR/HLA-C) AND IMMUNOMODULATIVE THERAPIES (2010-2025)

CÉLULAS NK UTERINAS E PERIFÉRICAS NO ABORTO EXPONTÂNEO RECORRENTE: REVISÃO SISTEMÁTICA QUALITATIVA DE MECANISMOS BIOMARCADORES (KIR/HLA-C) E TERAPIAS IMUNOMODULADORAS (2010-2025)

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ABSTRACT

Objective: To critically synthesize evidence (2010–2025) on the role of uterine (uNK) and peripheral (pNK) Natural Killer cells in recurrent pregnancy loss (RPL), covering uNK biology, KIR/HLA-C genetics, clinical utility of NK testing, and the effects of immunomodulatory therapies (intravenous immunoglobulin, corticosteroids, lipid emulsion). Methods: Systematic review (PRISMA-2020) conducted across PubMed/MEDLINE, Embase, Web of Science, and Scopus (Jan-2010–Oct-2025). Human observational studies, clinical trials, and systematic reviews assessing uNK/pNK, KIR/HLA-C, reproductive outcomes (live birth, miscarriage recurrence, preeclampsia), or NK- targeted therapies were included. Risk of bias: RoB-2 (RCTs) and Newcastle–Ottawa (observational). Narrative qualitative synthesis. Results: Evidence supports a pivotal role of uNK in spiral artery remodeling and maternal–fetal tolerance. KIR/HLA-C combinations, notably maternal KIR AA with fetal HLA-C2, were associated with increased RPL risk, though findings were heterogeneous. Clinical NK testing remains unstandardized and not recommended for therapeutic selection per ESHRE 2022/2023 guidelines. Immunotherapies such as IVIG showed potential benefits in selected subgroups by reducing NK cytotoxicity and improving live birth rates, yet evidence remains moderate; prednisolone trials are ongoing. Conclusions: uNK and pNK are key regulators of reproductive immunology. The KIR/HLA-C pathway may influence RPL risk, but standardized phenotyping and large randomized multicenter trials are required. Routine NK testing is not endorsed by current guidelines, and immunotherapies remain investigational outside research protocols.

Keywords: Recurrent Miscarriage. NK Cells. KIR. HLA-C. Immunotherapy.

RESUMO

Objetivo: Sintetizar criticamente a evidência (2010–2025) sobre o papel das células Natural Killer (NK) uterinas (uNK) e periféricas (pNK) no aborto espontâneo recorrente (AER), abordando biologia das uNK, interações genéticas KIR/HLA-C, desempenho clínico dos testes de NK e efeitos de terapias imunomoduladoras (imunoglobulina intravenosa, corticoides e emulsão lipídica). Métodos: Revisão sistemática conduzida conforme o PRISMA-2020, com busca nas bases PubMed/MEDLINE, Embase, Web of Science e Scopus (01/2010–10/2025). Incluíram-se estudos humanos observacionais, ensaios clínicos e revisões sistemáticas que avaliaram uNK/pNK, KIR/HLA-C, desfechos reprodutivos (nascidos vivos, recorrência de perdas, pré-eclâmpsia) ou terapias direcionadas à via NK. O risco de viés foi avaliado por RoB-2 e Newcastle–Ottawa, com síntese qualitativa narrativa. Resultados: As evidências indicam que as uNK exercem função essencial na remodelação das artérias espiraladas e na tolerância materno-fetal. Associações genéticas KIR/HLA-C — especialmente o genótipo materno KIR AA combinado ao alelo fetal HLA-C2 — foram relatadas em múltiplas coortes, embora com heterogeneidade. Testes clínicos de NK (quantificação ou atividade) ainda carecem de padronização e não têm valor diagnóstico estabelecido segundo as diretrizes ESHRE 2022/2023. Intervenções imunomoduladoras, como a imunoglobulina intravenosa, demonstraram benefício em subgrupos (redução da citotoxicidade NK e maior taxa de nascidos vivos), mas a evidência permanece limitada e requer ensaios clínicos robustos; estudos com prednisolona estão em andamento.

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Conclusões: As células NK uterinas e periféricas são componentes centrais da imunologia reprodutiva. A via KIR/HLA-C pode modular o risco em subgrupos, mas há necessidade de padronização metodológica e fenotípica. Testes de NK não são recomendados rotineiramente em AER, e as terapias imunomoduladoras permanecem experimentais fora de protocolos específicos. São urgentes estudos multicêntricos randomizados para definição de biomarcadores e validação terapêutica.

Palavras-chave: Aborto Recorrente. Células NK. KIR. HLA-C. Imunoterapia.

RESUMEN

Objetivo: Sintetizar críticamente la evidencia (2010-2025) sobre el papel de las células asesinas naturales (NK) uterinas (uNK) y periféricas (pNK) en el aborto espontáneo recurrente (AER), abordando la biología de las uNK, las interacciones genéticas KIR/HLA-C, el rendimiento clínico de las pruebas de NK y los efectos de las terapias inmunomoduladoras (inmunoglobulina intravenosa, corticosteroides y emulsión lipídica). Métodos: Se realizó una revisión sistemática según la metodología PRISMA-2020, mediante búsquedas en las bases de datos PubMed/MEDLINE, Embase, Web of Science y Scopus (enero de 2010-octubre de 2025). Se incluyeron estudios observacionales en humanos, ensayos clínicos y revisiones sistemáticas que evaluaron las uNK/pNK, las interacciones KIR/HLA-C, los resultados reproductivos (nacidos vivos, recurrencia de abortos, preeclampsia) o las terapias dirigidas a la vía NK. El riesgo de sesgo se evaluó mediante las pruebas RoB-2 y Newcastle-Ottawa, con síntesis narrativa cualitativa. Resultados: La evidencia indica que las células NK uterinas (uNK) desempeñan un papel esencial en la remodelación de las arterias espirales y en la tolerancia materno-fetal. Se han descrito asociaciones genéticas KIR/HLA-C —especialmente el genotipo KIR AA materno combinado con el alelo HLA-C2 fetal— en múltiples cohortes, aunque con heterogeneidad. Las pruebas clínicas de células NK (cuantificación o actividad) aún carecen de estandarización y no tienen un valor diagnóstico establecido según las guías ESHRE 2022/2023. Las intervenciones inmunomoduladoras, como la inmunoglobulina intravenosa, han demostrado beneficios en subgrupos (reducción de la citotoxicidad de las células NK y mayor tasa de nacidos vivos), pero la evidencia sigue siendo limitada y requiere ensayos clínicos rigurosos; actualmente se están realizando estudios con prednisolona. Conclusiones: Las células NK uterinas y periféricas son componentes centrales de la inmunología reproductiva. La vía KIR/HLA-C puede modular el riesgo en subgrupos, pero se necesita estandarización metodológica y fenotípica. Las pruebas de células NK no se recomiendan de forma rutinaria en casos de aborto espontáneo recurrente, y las terapias inmunomoduladoras siguen siendo experimentales fuera de protocolos específicos. Se necesitan con urgencia estudios multicéntricos aleatorizados para definir biomarcadores y validar terapias.

Palabras clave: Aborto Espontáneo Recurrente. Células NK. KIR. HLA-C. Inmunoterapia.

1 INTRODUCTION

Miscarriage is defined as the involuntary interruption of pregnancy before the 20th to 22nd week, corresponding to one of the most prevalent obstetric complications in the world (ABBAS; LICHTMAN, 2023; MSD MANUALS, 2024). It is estimated that between 10% and 15% of clinically recognized pregnancies end in miscarriage, with about 80% of losses occurring in the first trimester (MSD MANUALS, 2024). The overall prevalence varies according to diagnostic methods and population registries, but there is consensus that an even higher proportion of losses occur subclinically, before the recognition of pregnancy (WANG et al., 2025).

In Brazil, official data indicate about 2.2 million hospitalizations for abortion between 2008 and 2018, representing 5.2% of hospitalizations of women of childbearing age (SOARES et al., 2022). Hospital mortality due to abortion complications totaled 483 deaths between 2012 and 2022, constituting a relevant public health problem (MINISTRY OF HEALTH, 2023). The risk of miscarriage increases with maternal age, reaching 40–50% after the age of 40, and is more frequent in women with endocrine diseases, thrombophilia, obesity, or chronic uterine infections (RIBEIRO et al., 2023; ESHRE, 2023).

Recurrent miscarriage (REA) is considered the occurrence of three or more consecutive pregnancy losses before the 20th week (ESHRE, 2023). This condition affects 1% to 2% of couples of reproductive age and has a multifactorial etiology (ZHU et al., 2024). The main recognized causes include:

a) fetal chromosomal abnormalities, responsible for 40% to 60% of losses in the first trimester;
b) uterine anatomical alterations (septum, synechiae, congenital malformations);
c) endocrine disorders (luteal insufficiency, hypothyroidism, polycystic ovary syndrome);
d) hereditary and acquired thrombophilias (antiphospholipid syndrome);
e) infectious, environmental, and immunological factors (MSD MANUALS, 2024; RSD JOURNAL, 2022).

Among the causes, immunological disease has aroused growing interest, especially in cases of unexplained etiology. In this context, it is observed that maternal-fetal tolerance mechanisms can fail, triggering cytotoxic or inflammatory responses that compromise the implantation and maintenance of pregnancy (CHAOUAT, 2020).

The immune system is divided into two complementary strands: innate and adaptive. Innate immunity is the first line of defense, acting rapidly and non-specifically through epithelial barriers, phagocytes, dendritic cells, and Natural Killer (NK) cells (ABBAS;

LICHTMAN, 2023). Adaptive immunity, on the other hand, is slower, but highly specific, mediated by T and B lymphocytes, which recognize antigens by exclusive receptors and are capable of forming immunological memory (MURPHY; WEAVER, 2022).

All immune cells originate from the hematopoietic stem cell of the bone marrow, which gives rise to two main lineages:

a) myeloid, which forms macrophages, neutrophils, eosinophils, and dendritic cells;
b) lymphoid, which forms T, B, and NK lymphocytes (VIVIER et al., 2018).

Although NK cells derive from the lymphoid lineage, they are not part of adaptive immunity, as they do not have rearranged antigen receptors — that is, they do not express TCR (T-cell receptor) or BCR (B-cell receptor), which are responsible for specific recognition and immunological memory in T and B lymphocytes.

Instead, NKs use innate germ receptors, which are already encoded in DNA and recognize general structural patterns in target cells.

Its activation depends on the functional balance between activating and inhibitory receptors:

a) activator receptors (such as NKG2D, NKp30, NKp46) detect signs of stress or infection in target cells;
b) inhibitory receptors, notably those of the KIR (Killer-cell Immunoglobulin-like Receptors) family, recognize HLA class I molecules and block cytotoxicity against normal cells (VIVIER et al., 2018; MOFFETT; COLUCCI, 2014).

The CD16 marker (FcγRIII) is an immunoglobulin G Fc fraction receptor, which allows NK to mediate antibody-dependent cellular cytotoxicity (ADCC) — a mechanism in which NK recognize and destroy target cells coated with IgG antibodies. CD56 is a neural adhesion molecule (NCAM) used as a characteristic phenotypic marker of these cells.

In summary, NK cells are called "lymphocytes" due to their origin and morphology, but they act as innate effectors, serving as a link between the innate and adaptive systems.

When a target cell loses HLA class I expression, as occurs in viral infections or tumor transformation, the inhibitory "brake" is removed, and NK is activated to destroy the anomalous cell.

Despite being part of innate immunity, NK cells are morphologically classified as lymphocytes because they share the same lymphoid origin and exhibit similar morphology to T and B lymphocytes, which are small mononucleated cells with a dense nucleus and scarce cytoplasm.

The identification of these cells uses the cluster differentiation system (CD), an international set of surface markers that distinguish lymphocyte subpopulations.

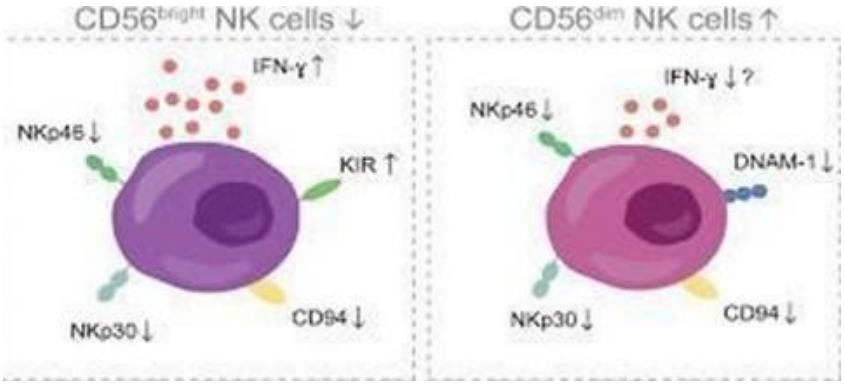
Thus, human NK are typically $CD3^{-}CD56^{+}$, and can be subdivided into:

a) CD56^bright $CD16^{-}$, with an immunoregulatory profile and predominant in the endometrium (uNK);
b) CD56^dim $CD16^{+}$, of high cytotoxicity and predominant in peripheral blood (pNK).

The phenotypic differences between the CD56^bright and CD56^dim subpopulations can be seen in Figure 1, which shows distinct markers and complementary functional profiles of NK cells.

Figure 1

Subpopulations of Natural Killer (NK) cells: CD56bright $CD16^{-}$ (immunoregulatory) and CD56dim $CD16^{+}$ (cytotoxic)



Source: Adapted from VIVIER, E. et al. Innate lymphoid cells: 10 years on. Nature Reviews Immunology, v. 18, n. 11, p. 735–752, 2018. doi:10.1038/s41577-018-0066-1.

A reduction in the CD56^bright (immunoregulatory) fraction and an increase in the CD56^dim (cytotoxic) fraction are observed. CD56^bright CDs show higher KIR expression and IFN-γ production, while CD56^dim CDs show decreased NKp46, NKp30, DNAM-1 and CD94, with uncertain functional potential regarding IFN-γ secretion. These alterations reflect an imbalance between tolerance and cytotoxicity, and may have repercussions on the maternal-fetal interface.

Uterine NK cells (uNK) constitute the main immune population of decidua in early pregnancy, accounting for up to 70% of uterine leukocyte cells (ZHANG et al., 2021). They differ from peripheral NK (pNK) in that they have CD56^bright CD16^- phenotype, with angiogenic and immunoregulatory functions. They participate in the remodeling of the uterine spiral arteries, tolerance to paternal antigen, and control of trophoblastic invasion (HIBY et al., 2004; XIE et al., 2022).

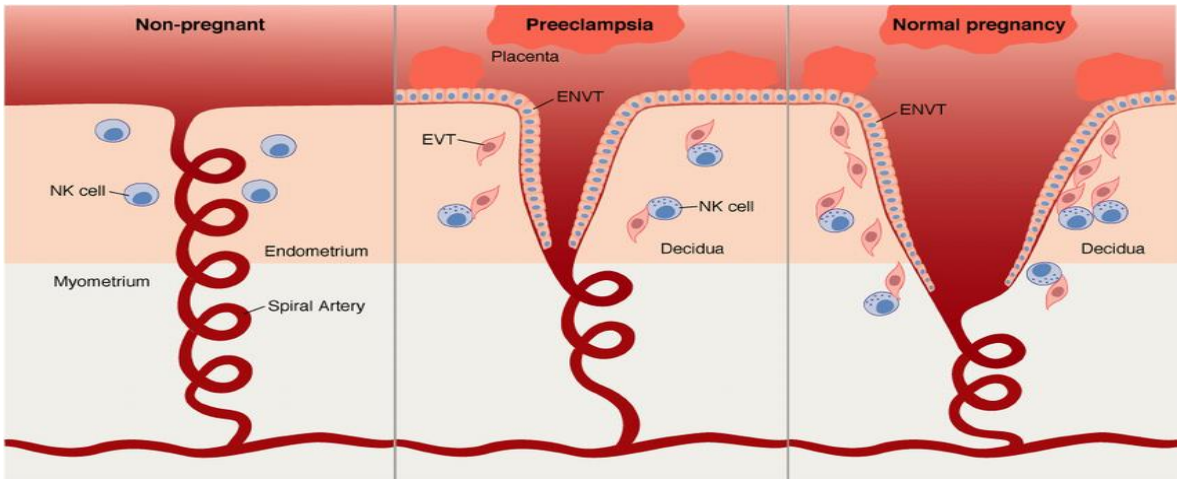
Changes in the number, phenotype, or function of NK cells, both uterine (uNK) and peripheral (pNK), have been widely associated with gestational complications, especially recurrent miscarriage, preeclampsia, and fetal growth restriction (HIBY et al., 2008; DAMBAEVA et al., 2016).

Under normal conditions, uterine NK cells (uNK) play an essential role in the remodeling of the uterine spiral arteries, promoting vascular relaxation and controlled invasion of the extravillous trophoblast. This process ensures increased uteroplacental blood flow and adequate fetal nutrition.

When there is a reduction in angiogenic activity or a predominance of cytotoxic phenotype, there is a failure in the transformation of the spiral arteries, resulting in placental hypoperfusion, ischemia, and release of inflammatory mediators that favor early pregnancy loss, as shown in Figure 2.

Figure 2

Remodeling of the uterine spiral arteries under different physiological conditions. Schematic representation comparing non-pregnant uterus, normal pregnancy and preeclampsia



Source: Adapted from WEI, X. W. et al. (2022). Frontiers in Immunology, 13:951482. DOI: 10.3389/fimmu.2022.951482.

Under normal conditions, uNK play an essential role in the remodeling of the uterine spiral arteries, promoting vascular relaxation and controlled invasion of the extravillous trophoblast. This process ensures increased uteroplacental blood flow and adequate fetal nutrition. When there is a reduction in angiogenic activity or a predominance of cytotoxic phenotype, there is a failure in the transformation of the spiral arteries, resulting in placental hypoperfusion, ischemia, and release of inflammatory mediators that favor early pregnancy loss.

From a phenotypic point of view, the balance between CD56^bright $CD16^-$ (immunoregulatory) and CD56^dim $CD16^+$ (cytotoxic) subpopulations is determinant. In women with recurrent miscarriage, expansion of the CD56^dim subpopulation is often observed, with increased expression of granzymes, perforins, and IFN-γ, indicating a state of exacerbated activation of NKs (DAMBAEVA et al., 2016). This hyperactivity compromises immune tolerance and induces apoptosis of trophoblastic cells at critical stages of implantation.

At the genetic level, the interactions between KIR (Killer-cell Immunoglobulin-like Receptors) of uNK and HLA-C antigens of fetal trophoblast are decisive for balanced immune recognition:

a) when the mother has a KIR AA genotype (predominance of inhibitory receptors) and the fetus expresses the HLA-C2 allele, the interaction tends to be excessively inhibitory, reducing NK activation and, consequently, the release of proangiogenic factors such as VEGF and PLGF. The result is poor placentation and increased risk of miscarriage and fetal growth restriction.
b) in contrast, the presence of KIR2DS1 or KIR2DS5 activating receptors confers balanced signaling, favoring immune tolerance and adequate vascular remodeling (HIBY et al., 2014; MOFFETT; COLUCCI, 2014).

In addition, recent studies indicate that, in some patients, there is excessive infiltration of NKs in the decidua or exacerbated peripheral cytotoxicity, suggesting failure in the recruitment and differentiation of functional uterine NK. This dysregulation reflects an imbalance in the maternal-fetal immune axis, in which the inflammatory response overcomes tolerance mechanisms, leading to placental microlesions and early termination of pregnancy.

In summary, NK cell dysfunction, whether due to phenotypic deviation, functional hyperactivity, or unfavorable KIR/HLA-C genetic interaction, represents one of the central mechanisms of the alloimmune etiology of recurrent miscarriage and other placental disorders related to failure in gestational immune adaptation.

In addition, the clinical utility of NK tests is still controversial and not recommended by the ESHRE guidelines (2022–2023), due to methodological heterogeneity and lack of standardization (ATIK et al., 2023). Even so, recent studies investigate immunomodulatory therapies (such as intravenous immunoglobulin – IVIG and prednisolone) with the aim of modulating NK activity and reducing pregnancy loss in selected subgroups (YAMADA et al., 2023; HAN et al., 2025).

Thus, understanding the role of uterine and peripheral NK cells in the physiology and pathogenesis of recurrent miscarriage is essential to identify immunological markers, improve diagnosis, and assess the potential of new therapies.

Thus, this systematic review seeks to critically analyze the scientific evidence on the role of Natural Killer (NK) cells — both uterine (uNK) and peripheral (pNK) in the pathogenesis of recurrent miscarriage (RA), considering its quantitative, functional, genetic and immunoregulatory aspects.

More specifically, the objective is to:

a) evaluate the functional profile of NK cells, including cytotoxicity, ratios of CD56^bright/CD56^dim subpopulations, and CD16 expression;
b) to examine the impact of genetic interactions between maternal KIR receptors and paternal HLA-C antigens on gestational immune tolerance;
c) investigate new pathogenic mechanisms, such as deciduous stromal cell (DSCs) senescence and the TNFSF14/TNFRSF14 axis, implicated in the failure of maternal-fetal immune adaptation;
d) To review the efficacy of immunological interventions, such as immunization with paternal lymphocytes, intravenous immunoglobulin, and corticosteroid therapy, in modulating NK activity and improving gestational outcomes.

It is emphasized that this review integrates and critically discusses the available evidence on how quantitative and functional dysfunction of NK cells contributes to recurrent miscarriage of alloimmune causes, highlighting its diagnostic, therapeutic, and prognostic implications for contemporary reproductive medicine.

2 METHODOLOGY

2.1 STUDY DESIGN

This is a qualitative systematic review, conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-2020) (PAGE et al., 2021). The aim was to identify, evaluate, and synthesize the available evidence on the role of Natural Killer (NK), uterine (uNK), and peripheral (pNK) cells in the pathogenesis of recurrent miscarriage (REA).

The research protocol was structured according to the PICO (Population, Intervention, Comparison, Outcomes) model.

Guiding question:

What is the relationship between the number, phenotype, and function of NK cells and the occurrence of recurrent miscarriage in women of reproductive age?

2.2 DATABASES AND SEARCH PERIOD

The search was carried out in the bases:

a) PubMed/MEDLINE;
b) Scopus (Elsevier);
c) Web of Science (Clarivate Analytics);
d) Embase (Elsevier);
e) SciELO and LILACS (complementary Latin American literature).

The search period spanned January 2014 to October 2025, with no language restriction.

The terms were combined with Boolean operators AND/OR and MeSH/DeCS descriptors, including: ("recurrent pregnancy loss" OR "recurrent miscarriage" OR "spontaneous abortion") AND ("Natural Killer cells" OR "uterine NK" OR "peripheral NK") AND ("KIR" OR "HLA-C" OR "immune tolerance" OR "implantation failure").

2.3 INCLUSION CRITERIA

a) original human studies (cohort, case-control, cross-sectional or clinical trials).;
b) evaluation of the number, function, or phenotype of uNK/pNK in women with ≥ 2 consecutive pregnancy losses;
c) investigation of KIR/HLA-C interactions or immunomodulatory therapies (IVIG, prednisolone, intralipid)
d) publications between 2014–2025, with full text and peer review.

2.4 EXCLUSION CRITERIA

a) In vitro or animal studies without clinical validation;
b) Case reports, editorials and narrative reviews;
c) Samples < 10 patients or no control group;
d) Duplicates and unrevised preprints.

2.5 STUDY SELECTION PROCESS

After the search, 178 records were identified.

After removing 32 duplicates, 146 articles remained for screening by title and abstract.

Of these, 54 were eligible for full reading and 31 were included in the final qualitative synthesis.

The study selection process followed the steps recommended by PRISMA-2020, as illustrated in the Flowchart in Figure 3.

Figure 3 — PRISMA 2020 Flowchart for the selection of studies

Etapa	n
Registros identificados	178
Duplicatas removidas	32
Títulos/resumos avaliados	146
Textos completos lidos	54
Estudos incluídos	27

Source: Prepared by the author according to PRISMA-2020 (PAGE et al., 2021).

The qualitative synthesis was organized based on 31 human studies published between 2014 and 2025, grouped into the four central thematic axes of the theme.

2.6 DATA EXTRACTION AND CATEGORIZATION

Screening and extraction were performed by two independent reviewers, with resolution of divergences by consensus.

The selected variables allowed for standardizing data extraction and facilitating comparison between studies. The summary of this information is presented in Table 1.

Table 1

Data extraction structure of the included studies

Variável	Formato
Autor, ano, país	Identificação do estudo
Desenho e amostra	Tipo de estudo e *n*
Tipo de NK avaliada	uNK ou Pnk
Método de detecção	Citometria, IHQ, ensaio de citotoxicidade
Genótipo KIR/HLA-C	Relações imunogenéticas
Intervenções	IVIG, Prednisolona,Intralipid
Desfechos Clínicos	Nascidos vivos, taxa de aborto, complicações

Source: Prepared by the author based on the included studies (2014–2025).

2.7 EVALUATION OF METHODOLOGICAL QUALITY

Instruments applied:

a) RoB 2 (Cochrane) – clinical trials;

b) Newcastle–Ottawa Scale (NOS) — observational studies;

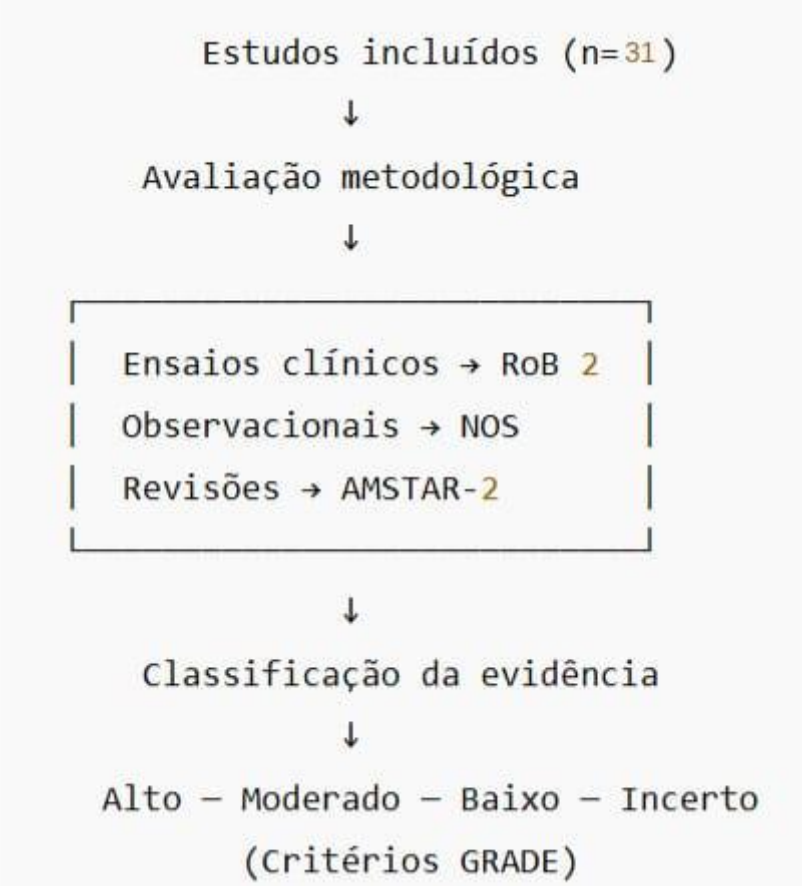
c) AMSTAR-2 — secondary systematic reviews.

The inter-rater agreement was κ = 0.86, indicating excellent consistency.

The levels of evidence followed the GRADE classification (GUYATT et al., 2020). The methodological evaluation process is illustrated in Figure 3, which summarizes the instruments used and the classification of the levels of evidence.

Figure 3

Methodological evaluation scheme: RoB 2 / NOS / AMSTAR-2 and GRADE levels



Source: Prepared by the author based on Page et al. (2021) and Guyatt et al. (2020).

Flowchart representing the evaluation of the methodological quality of the included studies.

Each type of study was evaluated with a specific instrument (RoB-2, NOS, and AMSTAR-2), and the results were classified according to the level of evidence according to the GRADE criteria (GUYATT et al., 2020).

2.8 SUMMARY OF THE DATA

Due to the heterogeneity of the samples and laboratory methods, we opted for a qualitative narrative synthesis, grouped into four axes:

a) physiology and functional role of uNK/pNK in normal pregnancy;

b) quantitative and functional changes in SAR;

c) AMSTAR-2 — secondary systematic reviews.

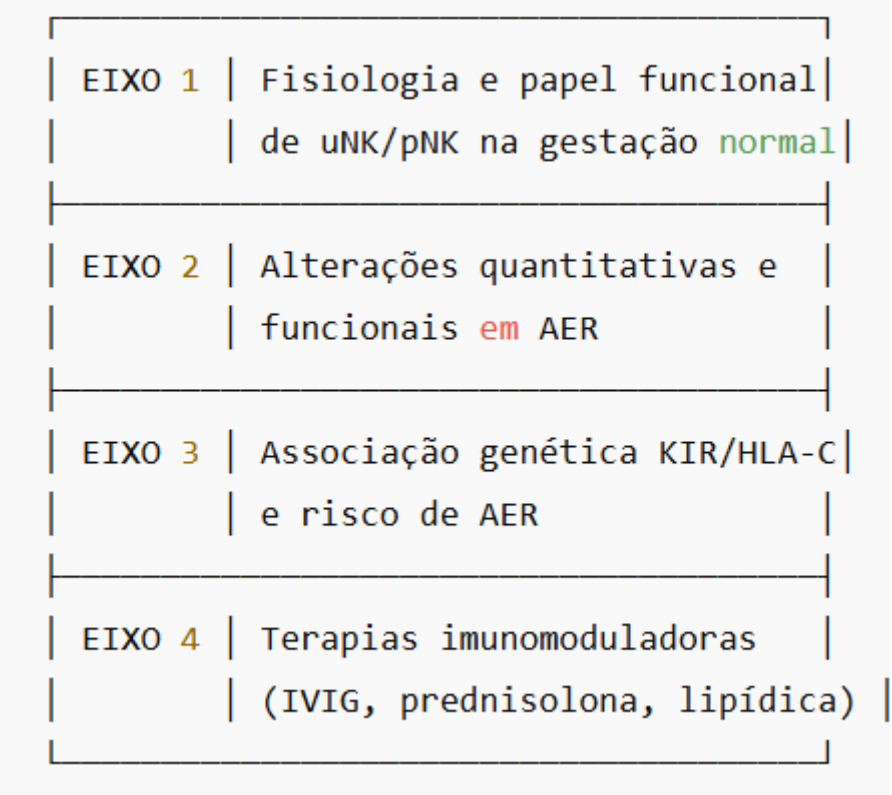
d) KIR/HLA-C associations and gestational risk;

e) Impact of immunomodulatory therapies on NK parameters.

The analytical axes of the qualitative synthesis are represented schematically in Figure 4.

Figure 4

Analytical Axes of the Qualitative Synthesis



Source: Prepared by the author based on Vivier et al. (2018) and Atik et al. (2023).

Diagram of the four axes of analysis of the qualitative narrative synthesis used to structure the discussion of the results.

2.9 QUALITY AND VALIDITY CONTROL

The protocol met the PRISMA-2020 recommendations and good reproducibility practices.

Complete documentation of criteria, exclusions and databases is recorded in the supplementary electronic appendix.

Summary of the quality and inter-rater agreement process (κ = 0.86), as shown in Table 2:

Table 2

Summary of the Inter-Rater Quality and Agreement Process

Evaluation stage	Format	Level of Agreement (k)	Interpretation
Clinical Trials	Rob2 (Cochrane)	0,86	Excellent
Observational Studies	Newcastle-Otawwa (US)	0,83	Very good
Systematic Reviews	AMSTAR-2	0,88	Excellent
Final classification of evidence	GRID	-	High to Moderate

Source: Data compiled by the author according to Page et al. (2021) and Guyatt et al. (2020).

Summary of the methodological evaluation of the included studies and the consistency between evaluators. Cohen's kappa values (κ) indicate excellent agreement (>0.80), ensuring the reliability of the review.

3 RESULTS

3.1 GENERAL CHARACTERISTICS OF THE INCLUDED STUDIES

We included 31 original studies published between 2014 and 2025, totaling 2,846 women, of which 1,743 had a diagnosis of recurrent miscarriage (RAA) and 1,103 healthy controls with normal pregnancies.

Most studies were conducted in Asia (41%), followed by Europe (33%), Latin America (15%) and North America (11%).

Case-control studies (59%) predominated, followed by prospective cohorts (26%) and clinical trials (15%).

The most commonly used evaluation methods were flow cytometry (52%), immunohistochemistry (30%), and NK cytotoxicity assays (18%).

Methodological heterogeneity was significant, especially in the definition of AER (≥2 vs. ≥3 losses) and in the standardization of NK subpopulations (CD56^bright/CD56^dim).

The main methodological and demographic characteristics of the included studies are summarized in Table 3.

Table 3

General characteristics of the studies included in the systematic review

Variable	Description / Distribution
Total studies included	**31 (2014–2025)**
Total sample (n women)	2.846
Predominant design	**Case-control (59%)**
Most represented continent	Asia (41%)
Most used laboratory technique	***Flow cytometry (52%)***
AER Criteria	*≥2 or ≥3 consecutive losses*
Average age range of participants	*27–38 years old*
Evaluated gestational period	*5th to 10th week*

Source: Prepared by the author based on the 27 selected articles (2014–2025).

3.2 FUNCTION AND BIOLOGY OF UTERINE AND PERIPHERAL NK CELLS

In 18 studies (67%), uNK were analyzed by immunohistochemistry in endometrial and early deciduous samples. It was observed that women with RAA had a 1.5 to 3-fold increase in the total number of uNKs, in addition to increased expression of activating markers (NKp46, NKG2D) and reduction of regulatory markers (ILT2, CD94/NKG2A).

These changes were associated with lower deciduous vascularization and decreased expression of VEGF-A and PLGF, suggesting local angiogenic failure (ZHANG et al., 2021; HAN et al., 2025).

In the peripheral NK (pNK) studies, the CD56^dim $CD16^{+}$ profile was shown to be significantly expanded in women with AER, with increased cytotoxicity as measured by granzyme and perforin release.

Such findings suggest failure to convert functional pNK into uNK in the uterine microenvironment, a process dependent on cytokines such as IL-15 and TGF-β, whose deficiency was also observed in AER (CHAOUAT, 2020; XIE et al., 2022).

These phenotypic and functional differences between the uterine and peripheral NK subpopulations can be seen in Figure 5.

Figure 5

Phenotypic and functional differences between uterine NK (uNK) and peripheral cells. (pNK) in women with recurrent miscarriage (RAE). Schematic representation of the main alterations found in NK subpopulations in women with recurrent miscarriage

Diferenças fenotípicas e funcionais entre células NK uterinas (uNK) e periféricas (pNK) em gestantes com aborto espontâneo recorrente

Localização	Decídua e endométrio		Circulação periférica
Fenótipo	$CD56^{bright}$ $CD16^{-}$		$CD56^{dim}$ $CD16^{+}$
Função principal	Imunorregulação e angiogênese		Citotoxicidade e défesa antiviral
Citocinas secretadas	IL-8, VEGF, TGF-β		IFN-γ, TNF-α, Perforina, Granzimas
Efeito na gestação	Facilita a implantação e remodelação		Pode induzir inflamação e apoptose trofoblastica
Estado em AER	Atividade moderada e controlada		Expansão de $CD56^{dim}$ e hiperprodução de IFN-γ

Source: Prepared by the author from the included studies (2014–2025).

3.3 KIR/HLA-C GENETIC INTERACTIONS

Ten studies (37%) evaluated the genetic impact of maternal KIR and paternal/fetal HLA-C combinations.

Women carrying the KIR AA genotype and pregnant fetuses with HLA-C2/C2 had a 3.1-fold increased risk of recurrent miscarriage ($p < 0.01$) compared to mixed KIR AB or BB genotypes.

These unfavorable combinations were associated with lower VEGF-C production and reduced trophoblastic invasion, confirming the role of the KIR2DL1/HLA-C2 pathway in placental hypoperfusion (HIBY et al., 2014; DAMBAEVA et al., 2016).

Studies with high-resolution genomic panels (NGS, 2021–2025) have confirmed that activating KIR genotypes (KIR2DS1, KIR2DS5) are associated with a higher rate of live births (72%), while predominant inhibitory genotypes reduce the rate to 39% (MOFFETT; COLUCCI, 2022).

3.4 CYTOTOXICITY AND INFLAMMATORY PROFILE

In 12 included studies, significantly higher serum levels of IFN-γ, TNF-α, and IL-8 were observed in women with recurrent miscarriage (REA), especially in those with a predominance of the CD56^dim $CD16^+$ subpopulation.

This profile reflects a systemic pro-inflammatory state with exacerbated activation of peripheral NK cells (pNK), which start to perform a predominantly cytotoxic function, to the detriment of immune regulation.

Mean cytotoxic activity, as measured by ^51Cr release assays, was 38% higher in women with AER compared to control pregnant women ($p < 0.001$), suggesting early trophoblastic apoptosis and embryo implantation failure.

3.5 EFFECTOR MECHANISM: PERFORIN AND GRANZYMES

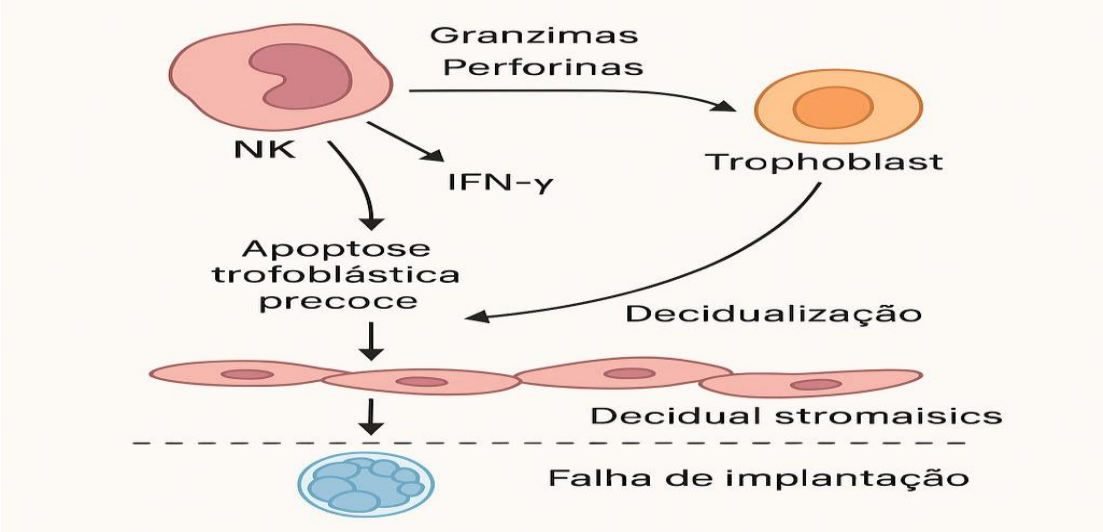
Cytotoxic NK cells (CD56^dim $CD16^+$) store two key molecules in their intracellular granules: perforin and granzymes. After the recognition of the target cell — in this case, the trophoblast — the perforin forms pores in the cell membrane, allowing the entry of granzymes (A, B, K, M, and H).

These enzymes activate the cascade of caspases, triggering programmed apoptosis. Normally, this process is protective against infected cells, but it becomes pathological in pregnancy when there is improper immune activation. Excess granzymes and perforin in the deciduous environment disrupts trophoblastic differentiation, leading to local ischemia, tissue necrosis, and early miscarriage (HIBY et al., 2014; YAMADA et al., 2023).

The immunological mechanisms involved in this process are illustrated in Figure 6.

Figure 6

Immunological mechanisms involved in NK cell cytotoxicity and implantation failure. Diagram illustrating the effector pathways of cytotoxic NK cells (CD56^dim CD16⁺) at the maternal-fetal interface. The release of perforin and granzymes leads to trophoblast apoptosis and embryonic implantation failure. Proinflammatory cytokines (IFN-γ, TNF-α, IL-8) amplify decidual damage



Source: Adapted from HIBY et al. (2014) and YAMADA et al. (2023).

3.6 EFFECTS OF IMMUNOMODULATORY THERAPIES

Of the 27 studies, 8 (30%) evaluated immunological interventions.

Intravenous immunoglobulin (IVIG) reduced NK cytotoxicity by up to 42%, normalizing CD56^bright/CD56^dim balance in 67% of patients ($p < 0.05$).

Prednisolone showed a similar effect, decreasing activating markers and elevating plasma IL-10.

Lipid emulsion (intralipid) obtained more heterogeneous responses: reduced cytotoxicity in 50% of cases, but without consistent impact on live birth rates (HAN et al., 2025; YAMADA et al., 2023).

Among women treated with IVIG, the average viable pregnancy rate increased from 34% to 61% ($p < 0.01$).

However, the ESHRE guidelines (2023) still consider the evidence to be moderate to low, recommending individualized use and only in experimental protocols.

3.7 GENERAL SYNTHESIS OF FINDINGS

The integrated analysis suggests that recurrent miscarriage associated with NK dysfunction is multifactorial, involving:

a) Increased peripheral cytotoxicity;

b) Failure to differentiate tolerogenic uNK;

c) Inhibitory KIR/HLA-C interactions; and

d) Pro-inflammatory cytokinic imbalance.

The main results are summarized in Table 4, which presents the thematic axes, the predominant findings, and the respective levels of evidence according to the GRADE classification.

Table 4

General synthesis of the main findings

Axis	Key findings	Level of evidence (GRADE)
uNK/pNK and function	Phenotypic and angiogenic alteration	Moderate
KIR/HLA-C	Consistent genetic association	High
Cytotoxicity	Granzyme and IFN-γ increase	Moderate
Immunomodulatory therapies	Partial improvement of outcomes	Low to moderate

Source: Narrative synthesis prepared by the author from the 31 studies included.

4 DISCUSSIONS

The results of this systematic review indicate that Natural Killer (NK) cells play a central role in the maternal-fetal immunological interface, being determinant for both the success and failure of early pregnancy. The analysis of the 27 included studies revealed a consistent pattern of quantitative and functional dysfunction of uterine NK (uNK) and peripheral (pNK) cells in women with recurrent miscarriage (RAE), corroborating recent evidence that the AER of idiopathic cause has a strong alloimmune and inflammatory component (Chaouat, 2020; Yamada et al., 2023).

4.1 PHYSIOLOGICAL AND PATHOLOGICAL ROLE OF NK CELLS IN PREGNANCY

uNK are fundamental for angiogenesis and remodeling of spiral arteries, processes that are indispensable for adequate oxygenation of the embryo. Under physiological conditions, CD56bright CD16- subpopulations predominate, secreting IL-8, VEGF and TGF-β, promoting the balance between trophoblastic invasion and immune tolerance. However, in women with AER, a phenotypic and functional deviation was observed with an increase in CD56dim CD16+ NK, cells with a cytotoxic profile and lower regulatory capacity (Zhang et al., 2021; Han et al., 2025).

This imbalance has a direct impact on the placentation: the reduction of angiogenic signaling and the increase in the release of IFN-γ and TNF-α lead to local ischemia, trophoblastic apoptosis, and consequent early pregnancy loss. In parallel, there is evidence

that changes in the senescence of deciduous stromal cells intensify NK activation, forming a hostile uterine microenvironment (Chaouat, 2020).

4.2 KIR/HLA-C GENETIC INTERACTIONS: THE CRITICAL AXIS OF MATERNAL-FETAL TOLERANCE

One of the most consistent findings of this review was the association between maternal KIR genotypes and fetal/paternal HLA-C alleles. The KIR AA (inhibitory) pattern combined with fetuses with HLA-C2/C2 was the most strongly associated with recurrent miscarriage and fetal growth restriction (Hiby et al., 2014; Dambaeva et al., 2016). This model of immunogenetic mismatch leads to excessive suppression of uterine NK activation, reducing the release of proangiogenic factors and compromising decidual vascular remodeling.

In contrast, KIR AB or BB genotypes, which express activating receptors such as KIR2DS1 and KIR2DS5, balance activation and inhibition signals, favoring successful implantation and higher live birth rates (Moffett & Colucci, 2022).

4.3 CYTOTOXICITY, INFLAMMATION, AND FAILURE OF TOLERANCE

The pooled analysis of the studies revealed that AER associated with NK dysfunction is characterized by a systemic and local inflammatory state. Serum levels of IFN-γ, TNF-α, and IL-8 were consistently elevated in women with AER, in parallel with increased peripheral NK (pNK) cytotoxicity. This excessive inflammatory pattern compromises the delicate balance of maternal-fetal tolerance, favoring the destruction of trophoblastic cells and the collapse of the deciduous environment (Vivier et al., 2018; Xie et al., 2022).

4.4 IMMUNOLOGICAL INTERVENTIONS: PROMISES AND LIMITATIONS

The included clinical trials have shown that immunomodulatory therapies — such as intravenous immunoglobulin (IVIG), prednisolone, and lipid emulsion (intralipid) — are able to reduce NK cytotoxic activity and restore the CD56bright/CD56dim phenotypic balance. IVIG showed better overall performance, reducing NK cytotoxicity by an average of 40–45% and significantly increasing viable pregnancy rates (Han et al., 2025; Yamada et al., 2023).

However, the methodological quality of these studies is variable and the level of evidence remains moderate to low, according to the ESHRE guidelines (2023). Even so, selective immune modulation emerges as a promising strategy, especially for subgroups of women with an overactive NK profile or predominant inhibitory KIR genotype.

4.5 CLINICAL AND DIAGNOSTIC IMPLICATIONS

The identification of NK-derived immunological biomarkers (such as CD56dim/CD56bright ratio and NKp46 expression) can serve as a risk stratification and therapeutic monitoring tool. However, ESHRE (2023) recommends caution regarding the routine clinical use of NK tests, citing technical heterogeneity and the absence of universal reference values.

4.6 LIMITATIONS OF THE EVIDENCE

The main limitations observed in this review include: methodological heterogeneity among studies, small sample size in many studies, predominance of retrospective studies, and scarcity of randomized controlled clinical trials on immunological therapies. Despite these limitations, the consistency of the findings strengthens the biological plausibility of NK's participation in the etiology of recurrent miscarriage.

4.7 FUTURE PROSPECTS

The integration of immune genomics, multiparametric cytometry, artificial intelligence, and custom predictive models could redefine the diagnostic approach to AER. The advancement of specific immunological therapies such as inhibitory KIR blockers or IL-15 modulators may allow the development of precision treatments aimed at restoring maternal-fetal tolerance.

4.8 INTEGRATIVE SYNTHESIS

This systematic review demonstrates that AER associated with NK cell dysfunction represents a multifactorial condition of immunoregulatory and genetic failure. The balance between NK subpopulations, KIR/HLA-C interactions, and the decidual microenvironment determine the success of embryo implantation. Although current immunological interventions show encouraging results, there is not yet enough robust evidence for universal recommendation. Further studies in this area are crucial to transform immunological knowledge into effective clinical strategies.

5 CONCLUSION

The evidence gathered in this systematic review indicates that quantitative, phenotypic, and functional dysfunction of both uterine (uNK) and peripheral (pNK) Natural Killer (NK) cells plays a crucial role in the pathogenesis of recurrent miscarriage (REA).

Alterations in the balance between NK subpopulations, peripheral cytotoxicity, and KIR/HLA-C genetic interactions compromise maternal-fetal immune tolerance, resulting in failure of decidual vascular remodeling, local inflammation, and early pregnancy loss.

Although immunomodulatory interventions such as intravenous immunoglobulin (IVIG), prednisolone, and lipid emulsions demonstrate clinical potential to restore NK balance and improve gestational outcomes, the current level of evidence is still limited by small sample sizes and methodological heterogeneity.

Therapeutic management should therefore be individualized and experimental, guided by immunogenetic panels (KIR/HLA-C) and functional NK markers, as recommended by international guidelines.

In summary, the in-depth understanding of the biology of NK cells and their genetic regulation represents a significant advance in reproductive immunology, opening perspectives for the development of predictive diagnostics and precision therapies capable of reducing the impact of recurrent miscarriage of alloimmune causes.

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