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Uterine natural killer cells: from foe to friend in reproduction

Indra Díaz-Hernández[1], Diana Alecsandru[2], Juan Antonio García-Velasco[2], and Francisco Domínguez[1,*]

[1]Fundación IVI, Innovation-IIS La Fe, Valencia 46026, Spain [2]Department of Immunology and Department of Reproductive Endocrinology and Infertility, Instituto Valenciano de Infertilidad-Madrid, Rey Juan Carlos University (IVI), Madrid 28023, Spain

*Correspondence address. Fundación IVI, Innovation-IIS La Fe, Avda. Fernando Abril Martorell 106, Torre A, 1° 1.23, Valencia 46026, Spain. E-mail: Francisco.Dominguez@ivirma.com https://orcid.org/0000-0002-7620-8081

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BACKGROUND: Recurrent miscarriage and pre-eclampsia are common reproductive disorders, but their causes are often unknown. Recent evidence has provided new insight into immune system influences in reproductive disorders. A subset of lymphocytes of the innate immune system known as uterine natural killer (uNK) cells are now recognized as fundamental to achieving embryo implantation and successful pregnancy, but were initially attributed a bad reputation. Indeed, immune therapies have been developed to treat the 'exaggerated' immune response from uNK cells. These treatments have been based on studies of peripheral blood natural killer (pbNK) cells. However, uNK cells and pbNK cells have different phenotypic and functional characteristics. The functions of uNK cells are closely related to their interactions with the extravillous trophoblast cells (EVTs) and spiral arteries, which underlie an essential role in regulating vascular function, controlling trophoblast invasion and promoting placental development. EVTs express MHC molecules of class I HLA-C/E/G/F, while uNK cells express, among other receptors, killer cell immunoglobulin-like receptors (KIRs) that bind to HLA-C or CD94/NKG2A inhibitory receptors, and then bind HLA-E. Associations of certain KIR/HLA-C combinations with recurrent miscarriage, pre-eclampsia, and foetal growth restriction and the interactions between uNK cells, trophoblasts and vascular cells have led to the hypothesis that uNK cells may play a role in embryo implantation.

OBJECTIVE AND RATIONALE: Our objective was to review the evolution of our understanding of uNK cells, their functions, and their increasingly relevant role in reproduction.

SEARCH METHODS: Relevant literature through June 2020 was retrieved using *Google Scholar* and *PubMed*. Search terms comprised uNK cells, human pregnancy, reproductive failure, maternal KIR and HLA-C, HLA-E/G/F in EVT cells, angiogenic cytokines, $CD56^{+}$ NK cells, spiral artery, oestrogen and progesterone receptors, KIR haplotype and paternal HLA-C2.

OUTCOMES: This review provides key insights into the evolving conceptualization of uNK cells, from their not-so-promising beginnings to now, when they are considered allies in reproduction. We synthesized current knowledge about uNK cells, their involvement in

reproduction and their main functions in placental vascular remodeling and trophoblast invasion. One of the issues that this review presents is the enormous complexity involved in studying the immune system in reproduction. The complexity in the immunology of the maternal–foetal interface lies in the great variety of participating molecules, the processes and interactions that occur at different levels (molecular, cellular, tissue, etc.) and the great diversity of genetic combinations that are translated into different types of responses.

WIDER IMPLICATIONS: Insights into uNK cells could offer an important breakthrough for ART outcomes, since each patient could be assessed based on the combination of HLA and its receptors in their uNK cells, evaluating the critical interactions at the materno–foetal interface. However, owing to the technical challenges in studying uNK cells *in vivo*, there is still much knowledge to gain, particularly regarding their exact origin and functions. New studies using novel molecular and genetic approaches can facilitate the identification of mechanisms by which uNK cells interact with other cells at the materno–foetal interface, perhaps translating this knowledge into clinical applicability.

Key words: uterine natural killer cells / killer cell immunoglobulin-like receptors / HLA-C / human pregnancy / materno–foetal interface / immunotherapies

Introduction

During pregnancy, remodeling of the maternal uterine spiral arteries is a key step for normal foetal growth and development (Kam *et al.*, 1999). For proper development of the placenta, the cells of the foetal trophoblast must infiltrate the decidua and transform the placental bed spiral arteries during the first weeks of pregnancy. This ensures sufficient oxygen and nutrient supply to promote normal foetal growth and development. The invasion of the uterus by the trophoblast should not be excessive, since this would generate risks for the mother. However, poor arterial transformation would deprive the feto-placental unit: when vascular remodeling is deficient, so is the placental blood supply, which could lead to pregnancy complications such as pre-eclampsia, maternal hypertension, foetal growth restriction (FGR), preterm labor, or late miscarriage (Brosens, 1977; Khong *et al.*, 1986; Brosens *et al.*, 2011). Therefore, this process must be well controlled and balanced.

Villous trophoblasts and extravillous trophoblasts (EVTs) are the two main subpopulations of trophoblast cells. The syncytiotrophoblast comes into contact with maternal blood, while EVTs invade the decidua, spiral arteries, and myometrium (King *et al.*, 1991; Genbacev *et al.*, 1992; Moffett and Loke, 2006; Chazara *et al.*, 2011). Few species have a placental invasion as deep and complex as humans (Carter and Pijnenborg, 2011). Invasion of the uterine wall by trophoblasts causes EVTs to surround the uterine arteries (Pijnenborg *et al.*, 1983). It was previously thought that EVTs replace the maternal endothelial cells of these arteries during remodeling. However, recent evidence now suggests this is not the case and that loss of endothelial cells is instead a transient process with regenerative capacity (Bulmer *et al.*, 2020).

These changes promote the increase in blood flow necessary for normal foetal growth and development (Moffett-King, 2002). Yet, infiltration of the foetal tissues creates an immunological dilemma because the fetus can express paternal antigens. The placenta could also be rejected by being recognized by the maternal immune system as non-self, similar to what can occur during transplant rejection (Trundley and Moffett, 2004). The immune system plays an essential role in establishing these maternal–foetal limits (Hiby *et al.*, 2010a).

In humans, T and B cells are relatively rare at the endometrial embryo implantation site, while the dominant lymphocyte population is represented by uterine natural killer (uNK) cells (King *et al.*, 1998). Although the exact role of uNK cells is not fully unravelled, the secretion of cytokines by these cells could be involved in remodeling the blood vessels and creating a propitious microenvironment for the fetus (Croy *et al.*, 2003). Peripheral blood natural killer (pbNK) cell cytotoxicity and cytokine production are essential in defending the body against viruses or even controlling the early spread of tumours (Moffett and Shreeve, 2015). Although their name is reminiscent of the ability of pbNK cells to destroy other cells, uNK cells have a low cytotoxic response (King *et al.*, 1989). However, their name has prompted the idea that uNK cells could be responsible for miscarriages or failures after IVF treatments by executing their 'destructive' ability against the embryo (Quenby *et al.*, 1999; Tuckerman *et al.*, 2007). So far, there is no evidence that this occurs, but immune therapies targeting NK cells have been developed (Wong *et al.*, 2014). In fact, concerning statements have purported that NK cells can be so aggressive that they attack pregnancy by recognizing the fetus as a foreign body (Shehata, 2014; Alan, 2015). uNK cells do not come into direct contact with the fetus, but rather with the trophoblast (Moffett-King, 2002; Sharkey *et al.*, 2008). Despite the aforementioned differences between pbNK and uNK cells, some fertility clinics still perform blood tests to measure pbNK cell number and activity (Yamada *et al.*, 2003; Wang *et al.*, 2008). As a result of therapeutic trials, treatments such as steroids or intravenous immunoglobulins (IVIG), which are not risk-free for the mother or the fetus (Duhem *et al.*, 1994), are offered to women with infertility or other reproductive disorders (Nyborg *et al.*, 2014; Ehrlich *et al.*, 2019).

uNK cells differ from pbNK cells both in cell surface markers and functions (Caligiuri, 2008; Moffett and Shreeve, 2015). uNK cells have killer cell immunoglobulin-like receptors (KIRs), which are membrane receptors that bind to human leukocyte antigen (HLA)-C (classical MHC class I antigen) present on the EVTs. EVT cells also express the three non-classical MHC class I antigens, HLA-E, HLA-F, and HLA-G (Ishitani *et al.*, 2003; Apps *et al.*, 2008; Hackmon *et al.*, 2017). Recently, certain KIR/HLA-C combinations have been associated with an increased risk of recurrent miscarriage (RM), pre-eclampsia or foetal growth restriction (Hiby *et al.*, 2010a,b). The objective of this essay is to review (i) how research on uNK cells has evolved, (ii) knowledge of their functions and (iii) their increasingly relevant role in reproduction.

The origin of uNK cells

The study of what we know today as uNK cells has a long history. They were first described in the pregnant uterine mucosa 100 years

ago and were referred to as a *type of leukocyte restricted to the decidua* (Weill, 1921). The first descriptions of these cells were made in rodents, where they were called granulated metrial gland cells (Peel, 1989). In the 1980s, several articles reported these cells in the human endometrium, referring to them as large granular lymphocytes owing to their size and prominent cytoplasmic granules (Starkey *et al.*, 1988; King *et al.*, 1991), endometrial stromal granulocytes (Bulmer *et al.*, 1987) or NK cells in early pregnancy decidua (Manaseki and Searle, 1989). In some cases, they were identified as lymphoid cells. However, because their characteristics were more similar to those of NK cells, in the 1990s it was firmly established that these endometrial cells were a type of lymphocyte in the NK cell lineage (King *et al.*, 1991).

uNK cells present phenotypic and functional characteristics different from those of pbNK cells (Moffett-King, 2002). Further, because of differences in gene expression, uNK cells have been proposed to represent a distinct lineage of NK cells (Koopman *et al.*, 2003). uNK cells appear small and agranular in the pre-ovulatory proliferative phase; while in the post-ovulatory secretory phase, they proliferate, enlarge, and granulate (Spornitz, 1992). Depending on the relative expression of CD16 (also known as FcγRIII, low-affinity receptor for the Fc portion of IgG) and CD56 (adhesion molecule mediating homotypic adhesion), subpopulations of NK cells can be identified and classified (Cooper *et al.*, 2001; Mavilio *et al.*, 2005; Caligiuri, 2008). uNK cells express high levels of CD56 and are negative for CD16 ($CD56^{bright}$ $CD16^{-}$), whereas approximately 90% of pbNK cells express low levels of CD56 and are positive for CD16 ($CD56^{dim}CD16^{+}$) (Ritson and Bulmer, 1987; King *et al.*, 1991; Trundley and Moffett, 2004). These two NK subpopulations also show functional differences. $CD56^{dim}$ cells are more cytotoxic and have many cytolytic granules compared to $CD56^{bright}$ cells; $CD56^{bright}$ cells are the major cytokine-producing NK cells (Cooper *et al.*, 2001). uNK cells also have higher expression of CD56 (King *et al.*, 1991), are larger, and contain more cytolytic granules than $CD56^{bright}$ pbNK cells. uNK cells have been identified both in the endometrium of non-pregnant women and in the decidualized endometrium of pregnant women, varying in quantity throughout the menstrual cycle and during pregnancy (Bulmer *et al.*, 2010).

During the proliferative phase of the menstrual cycle, these $CD56^{+}$ cells increase in number and reach their maximum level in the late secretory phase, an increase that is maintained until the early stages of pregnancy (King *et al.*, 1991; Whitelaw and Croy, 1996). However, uNK cells are less concentrated in the endometrium of premenarchial and postmenopausal women (Hameed *et al.*, 1995; Kämmerer *et al.*, 2003). In the decidua, more than 70% of the leukocytes are $CD56^{+}$ and aggregate around glands and spiral arteries (Trundley and Moffett, 2004). When the trophoblast completes its invasion (around the 20th week of pregnancy), the number of uNK cells begins to decrease, constituting a much smaller lymphocyte population compared to that of the first trimester. Likewise, as will be described later, these changes in the number of uNK cells are related to fluctuations in the hormones that induce decidualization and chemokine expression (Red-Horse *et al.*, 2001; Jones *et al.*, 2004; Ordi *et al.*, 2006).

The origin of uNK cells is not yet clear. Their gene expression pattern differs remarkably from that of pbNK cells (Koopman *et al.*, 2003). In fact, according to their genetic profile, $CD56^{bright}$ pbNK cells are more similar to $CD56^{dim}$ pbNK cells than to $CD56^{bright}$ uNK cells (Koopman *et al.*, 2003). It is still unknown where and how their differentiation begins; if it is a local effect; if they are recruited by the endometrium; or if there are both local and distant events. Several hypotheses exist to explain how these cells originate (Fig. 1). uNK cells could be generated from pbNK cells, in which case the cells would differentiate in the uterus (King *et al.*, 1991). Since stromal cells are in close contact with uNK cells, they could play an important role during uNK cell differentiation in the uterus (Kitaya *et al.*, 2000, 2003; Gubbay *et al.*, 2002). Transforming growth factor beta (TGFβ) appears to promote the conversion of $CD16^{+}$ pbNK cells into $CD16^{-}$ cells, which would therefore resemble the phenotype of uNK cells (Keskin *et al.*, 2007). TGFβ has also been proposed to act on progenitor cells present in the decidua to direct their differentiation towards uNK cells (Keskin *et al.*, 2007). Chemokine production varies throughout the menstrual cycle, so chemokines are also suggested to play an essential role in recruiting these pbNK cells into the decidua (Hornung *et al.*, 1997; Kitaya *et al.*, 2003; Hannan *et al.*, 2004; Jones *et al.*, 2004): the latter hypothesis is reinforced by the expression of chemokine receptors in uNK cells (CXCR3 and CXCR4) that mediate the migration of pbNK cells to the decidua (Hanna *et al.*, 2003). Some evidence indicates that uNK cells could also originate from hematopoietic precursors already present in the endometrium (*in situ* differentiation) (Matsuura-Sawada *et al.*, 2005; Lynch *et al.*, 2007; Manaster *et al.*, 2008; Chiossone *et al.*, 2014).

uNK cells have also been proposed to proliferate directly in the uterus from precursor cells characterized by CD34 expression (Trundley and Moffett, 2004; Bulmer and Lash, 2005). Some studies using mouse models also suggest that uNK cells proliferate during decidualization from the local NK cells present in the decidua basalis and myometrium (Sojka *et al.*, 2018, 2019). Other studies support the possibility that the endometrium recruits hematopoietic stem cells and provides them with an optimal microenvironment for uNK cell differentiation and proliferation (Taylor, 2004; Keskin *et al.*, 2007). If pbNK cells were recruited in the decidua, owing to the functional and phenotypic differences between pbNK and uNK cells, pbNK cells must undergo greater differentiation to acquire the functional and phenotypic characteristics typical of uNK cells. Factors provided by the decidualized stroma, such as interleukin (IL)-11 and IL-15, seem to be involved in this phenotypic and functional differentiation process (Santoni *et al.*, 2008). IL-15 is a key cytokine in hematopoietic progenitor cell differentiation towards NK cells and its expression increases in the decidua (Becknell and Caligiuri, 2005). The increase of IL-15 in the decidua after fertilization is hypothesized to promote differentiation of these NK cells towards uNK cells.

Gene expression differences between pbNK and uNK cells also present the possibility that uNK cells represent a different lineage of NK cells, that is, uNK cells present in the decidua are derived from a different hematopoietic precursor (Koopman *et al.*, 2003). In fact, three subpopulations of uNK cells (uNK1, uNK2 and uNK3) were recently described based on differential expression of key genes and morphological differences (Vento-Tormo *et al.*, 2018). uNK1 cells express higher levels of KIRs and are the only uNK cells that express *LILRB1* (the receptor for HLA-G molecules). Only uNK1 and uNK2 cells express *NKG2C* (or *KLRC2:* killer cell lectin like receptor C2), *NKG2E* (or *KLRC3*) and *NKG2A* (or *KLRC1*). Additionally, uNK1 cells have more granules and a greater number of enzymes involved

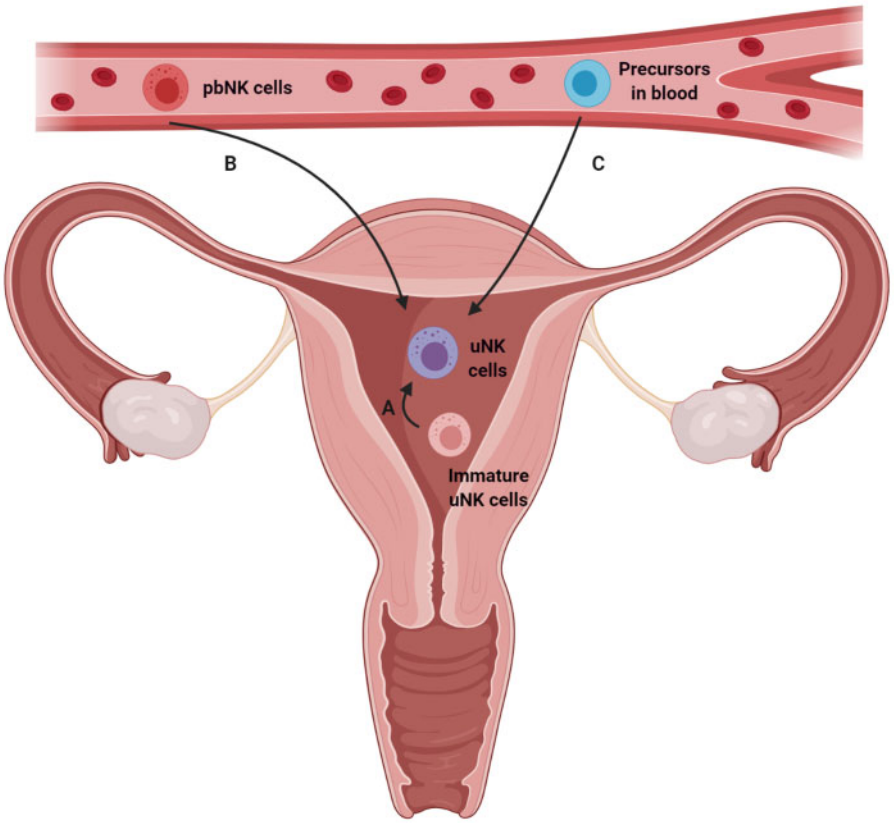

Figure 1. Possible origins of uterine natural killer cells. (**A**) Origin and proliferation of uterine natural killer (uNK) cells within the decidua itself. There is evidence suggesting that uNK cells could differentiate *in situ* from the local hematopoietic progenitors ($CD34^+$ precursors present in decidual tissues) (Eriksson *et al.*, 2004; Male *et al.*, 2010; Vacca *et al.*, 2011). The production of different factors and interleukin-15 in the endometrium could create an environment conducive to the development of uNK cells from lineage-negative (Lin^-) $CD34^+$ $CD45^+$ cells present in the decidua (Keskin *et al.*, 2007). (**B**) Recruitment of peripheral blood (pb)NK cells and their subsequent differentiation into uNK cells. The recruited pbNK cells have been proposed to be either $CD56^{bright}$ $CD16^-$ pbNK cells or $CD56^{dim}$ $CD16^+$ pbNK cells. (**C**) Precursors present in the peripheral blood that reach the uterus and differentiate, in the event that they derive from a different hematopoietic precursor (Keskin *et al.*, 2007). Created with BioRender.com.

in glycolysis than uNK2 and uNK3 cells. Together, these data suggest that uNK1 cells interact especially with EVT cells (Vento-Tormo *et al.*, 2018).

uNK cell regulation by steroid hormones

Given the association between the number of uNK cells and the menstrual cycle, sex steroid hormones (oestrogen and progesterone) seem to be responsible for regulating uNK cell proliferation in the late secretory and decidualized endometrium (Fig. 2). Stromal cells have also been proposed to be indirectly responsible for exerting a hormonal influence on uNK cells, since they express classical oestrogen and progesterone receptors (ER and PR, respectively) (King *et al.*, 1996). Furthermore, although uNK cells were thought to be devoid of steroid hormone receptors, they do express ER-b, which is a lower-affinity variant of the classical ER (Henderson *et al.*, 2003). On the other hand, when progesterone levels drop, so does the number of uNK cells, indicating that uNK cell number is dependent on progesterone (Acar *et al.*, 2011). uNK cells express both mRNA and protein for ERβ1 and glucocorticoid receptor (GR), but not for PR (Henderson *et al.*, 2003). Although pbNK cells express PR (especially $CD56^-$), uNK cells do not appear to express PR (Arruvito *et al.*, 2008). This suggests that the effect of progesterone on these cells must be indirect (King *et al.*, 1996) (Fig. 2). Stromal cells do have PR, so progesterone binding to PR triggers IL-15 secretion (Okada *et al.*, 2000). *In-vitro* studies showed that progesterone favours the production of IL-15 by endometrial stromal cells in the decidualization process. These results demonstrate the importance of IL-15 during the secretory phase and the early stages of pregnancy when the corpus luteum releases this hormone. Therefore, IL-15 could play an important role in the proliferation and differentiation of uNK cells and in the success of

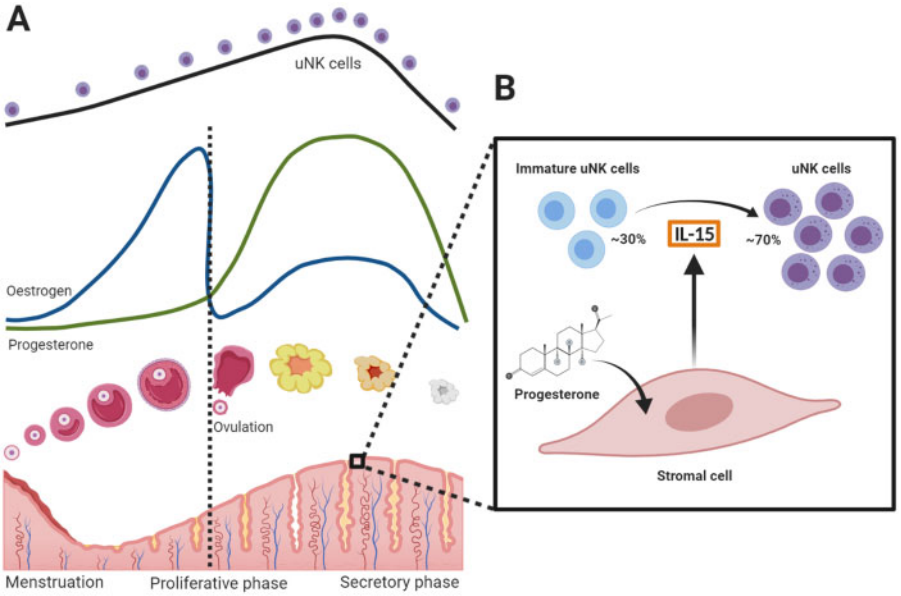

Figure 2. uNK cells throughout the menstrual cycle. (**A**) Variation in the number of uNK cells, physiological and hormonal changes throughout the menstrual cycle. Although uNK cells do not express progesterone receptor (PR), there is a clear relation between steroid sex hormones and uNK cell proliferation. Since the number of uNK cells reaches its peak in the secretory phase, where high production of progesterone also occurs, the effect of progesterone is believed to be indirect, through stromal cells. (**B**) The concentration of uNK cells increases considerably when passing from the proliferative phase to the secretory phase, going from constituting 30% of total lymphocytes in the endometrium at mid-secretory phase to reaching 70–80% of the decidual leukocytes (King *et al.*, 1991; Hanna *et al.*, 2006). The proliferation of these cells appears to be controlled by fluctuating hormonal levels throughout the menstrual cycle and pregnancy. Among the most studied factors and hormones are interleukin (IL)-15 and progesterone. In the secretory phase of the endometrium and the early stages of pregnancy, an increase in progesterone predominates and the expression of IL-15 is much higher than in the proliferative phase (Kitaya *et al.*, 2005). Therefore, progesterone has been proposed to act through stromal cells (which do express PR), which then release IL-15, a cytokine that could be responsible for the proliferation/differentiation of the uNK cells (Verma *et al.*, 2000). Created with BioRender.com.

pregnancy (Okada *et al.*, 2000). In fact, other *in-vitro* studies showed that IL-15 stimulates the proliferation of uNK cells without promoting cytolytic activity against trophoblasts (Verma *et al.*, 2000; Kitaya *et al.*, 2003). Furthermore, murine NK cell differentiation requires this IL-15 (Puzanov *et al.*, 1996).

Along with the other predecidual changes that occur in the endometrium during the secretory phase, uNK cells maintain arterial stability as long as the corpus luteum remains in the early decidua and will continue until the trophoblast-mediated arterial transformation occurs (Kam *et al.*, 1999). In the event of no pregnancy, the corpus luteum degenerates and the levels of progesterone and IL-15 decrease, causing uNK cells to disappear and the vascular collapse that leads to menstruation. Thus, it seems that the proliferation and maturity of uNK cells in a normal non-pregnant endometrium is also due to the influence of progesterone, which causes IL-15 trans-presentation from stromal cells (Wilkens *et al.*, 2013).

Since it has been proposed that NK cells could be recruited by the endometrium (Chantakru *et al.*, 2002), various chemokines that would induce such migration have been studied (Campbell *et al.*, 2001; Inngjerdingen *et al.*, 2001; Hanna *et al.*, 2003). Sex hormones can induce the expression of certain chemokines, such as CXCL10 (IP-10) and CXCL11 (I-TAC), in the human endometrium (Sentman *et al.*, 2004). Furthermore, both pbNK cells and uNK cells express high levels of specific receptors for these chemokines. Therefore, progesterone and oestradiol could regulate the expression of specific chemokines in the endometrium that, in turn, would be involved in the recruitment of NK cells in the uterus (explaining the increase in the number of uNK cells during the menstrual cycle) (Sentman *et al.*, 2004).

The functions of uNK cells: not so similar to those of pbNK cells

While pbNK cells play a role in the immune response against viruses, tumours or infections (Flaherty, 2012), uNK cells play a fundamental role during embryo implantation and trophoblast invasion, ensuring proper placentation through vascular remodeling (Pace *et al.*, 1989; Robson *et al.*, 2012; Xiong *et al.*, 2013). pbNK cells represent between 10% and 15% of the lymphocyte population in the peripheral blood and the two most relevant functions of pbNK cells are target-cell killing and cytokine production (Jonges *et al.*, 2001). As uNK cells

could affect hormonal regulation of the endometrium (Hickey et al., 2005), they have been proposed to be not only regulators of placentation, but also participants in endometrial cycle homeostasis and maintenance of the decidua (Wilkens et al., 2013). In the first weeks of pregnancy, uNK cells infiltrate and accumulate around spiral arteries. In both the first and second trimesters, the number of uNK cells is maintained. However, uNK cell numbers are significantly reduced in the third trimester of pregnancy, although a residual population of these cells is preserved (Williams et al., 2009). Therefore, uNK cells' most notable presence coincides with the invasion period of the trophoblast (King et al., 1998). The presence of uNK cells throughout the menstrual cycle suggests that a role in the collapse and endometrial renewal (Loke and King, 1995). However, these cells appear to have a broader role since $CD56^{+}$ $CD16^{-}$ uNK cells are the most abundant leukocyte population during the first trimester of human pregnancy (Moffett-King, 2002), representing approximately 70% of decidual leukocytes (Hiby et al., 2010a). There is evidence that uNK cells are likely involved in the regulation of placentation and trophoblast invasion (Hanna et al., 2006; Xiong et al., 2013; Moffett and Colucci, 2014). In addition to differentiating and proliferating in the implantation window (Pace et al., 1989), uNK cells populate the decidua immediately adjacent to the infiltrating EVTs and around the spiral arteries during early pregnancy (Xiong et al., 2013).

These uNK cells are also characterized by secreting several factors involved in vascular remodeling and angiogenesis (Chen et al., 2017a), an action that occurs when EVT invasion begins shortly after implantation (Robson et al., 2012). Therefore, uNK cells have been associated with this implantation process, and attributed with a role in establishing normal early placentation through vascular remodeling.

Women with heavy menstrual bleeding have altered endometrial vascular maturation. In fact, heavy menstrual bleeding has been linked to dysregulation of uNK cells (Biswas Shivhare et al., 2015). When comparing endometrial leukocyte populations throughout the menstrual cycle in patients with heavy menstrual bleeding compared to healthy controls, the $CD56^{+}$ uNK cell population shows the greatest alterations (Biswas Shivhare et al., 2015). Specifically, the proportion of $CD56^{+}$ cells significantly increases in the proliferative and early secretory phases yet decreases in the late secretory phase in patients with heavy menstrual bleeding compared to controls. These results are consistent with previous studies noting altered differentiation of vascular smooth muscle cells (VSMCs) in patients with menorrhagia (Shivhare et al., 2014) and could be related to reduced vascular development. Similarly, a positive relation also has been noted between the number of uNK cells and vascular maturation in women with RM (Quenby et al., 2009).

Inadequate placentation associated with poor vascular remodeling is the main characteristic of pre-eclampsia (Roberts, 2003). Therefore, the primary pathological defect in this disease is the failure of the transformation of the spiral arteries (Moffett-King, 2002; Pijnenborg, 2003). The relationship between uNK cells and the vascular remodeling necessary for the proper development of pregnancy indicates that one of the possible key factors that may be failing when pre-eclampsia develops is related to the uNK cells (Wallace et al., 2015; James-Allan et al., 2018). Furthermore, this altered vascular conversion and insufficient invasion of the uterine lining by trophoblasts (Burton et al., 2009) are considered to be the main defects in not only pre-eclampsia, but also in disorders such as FGR and RM (Arck and Hecher, 2013).

Trophoblast and uNK cell receptors: interactions at the materno–foetal interface

uNK cells are generally found most abundantly among trophoblast cells that invade the decidua (King et al., 1998). In fact, when an ectopic pregnancy occurs, or the embryo attaches to the scar tissue from a previous caesarean section, uncontrolled growth and invasion occur due to the absence of decidua. Therefore, the cells that make up the decidua (such as uNK cells) must exert control over the degree of placental invasion by interacting with the trophoblast ligands (Warren, 1995). NK cell receptors were discovered when they were found to be capable of killing major histocompatibility complex (MHC) class I negative tumour cells (Ljunggren and Kärre, 1990). Three groups of these NK cell receptors have been described (Yokoyama, 1993): the KIR family (Moretta et al., 1993), the C-type lectin family (CD94/NKGs) (Lazetic et al., 1996) and immunoglobulin-like transcripts (ILTs or LIRs) (Samaridis and Colonna, 1997). HLA-C binds to KIRs of NK cells, HLA-G binds to members of the leukocyte immunoglobulin-like receptor family (LILR) and KIRs, and HLA-E binds to CD94/NKG2 (Parham, 2004).

KIR receptors are defined as 'surface inhibitory receptors specific for allelic forms of HLA class I molecules, which are expressed by NK cells and a subset of T lymphocytes' (Moretta and Moretta, 2004). However, as will be seen later, uNK cells exert not only an inhibitory action, but also an activator action. KIR receptors help regulate NK cells recognizing 'normal' cells versus target cells (e.g. tumour or infected cells) by interacting with MHC class I molecules (Colonna and Samaridis, 1995; Wagtmann et al., 1995; Handgretinger et al., 2016).

KIRs are type I transmembrane glycoproteins (Wagtmann et al., 1997) encoded by a group of highly polymorphic genes located on human chromosome 19q13.4 (Faridi and Agrawal, 2011). There are 16 different KIR genes, identified as *KIR2D* if they encode two immunoglobulin-like extracellular domains, or as *KIR3D* if they encode three immunoglobulin-like extracellular domains. Likewise, genes are identified by whether the encoded cytoplasmic tail is long (*KIR2DL* and *KIR3DL*) or short (*KIR2DS* and *KIR3DS*) (Fig. 3) (Colonna and Samaridis, 1995; Biassoni et al., 1996). However, classification is generally done according to more functional terms. They are classified as haplotype A or B depending on the content of their encoding gene (Cooley et al., 2018). Although more than 1110 KIR alleles have been described (IPD—KIR Database Statistics | EBI, 2019), they are all classified into two main functional categories, activators (KIR B) or inhibitors (KIR A), depending on how they regulate the 'cytotoxic' and angiogenic behaviour of uNK cells (Vilches and Parham, 2002). The activating KIR receptors have a short (S) cytoplasmic tail (2DS, 3DS) while the inhibitors have a long (L) cytoplasmic tail (2DL, 3DL) (Hong et al., 2008). Therefore, this classification is determined by the type and number of genes that encode activating or inhibiting KIRs (Faridi and Agrawal, 2011). The KIR A haplotype has only seven KIR genes: *KIR3DL3-2DL3-2DL1-2DL4-3DL1-2DS4-3DL2*. The *KIR2DS4* gene is the only activator in the A haplotype, but is generally non-functional

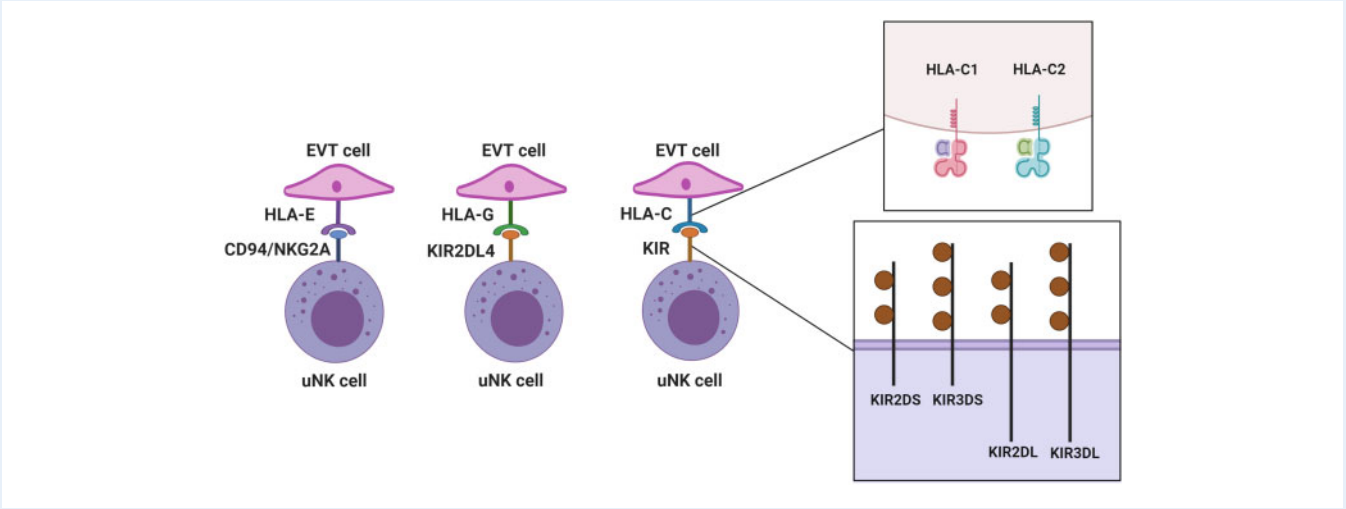

Figure 3. Representation of the receptors present in uNK cells that bind to HLA class I molecules. Extravillous trophoblast (EVT) cells express a unique combination of four major histocompatibility complex (MHC) class I molecules: HLA-C, HLA-E, HLA-F and HLA-G. These include three non-classical (HLA-E, HLA-F and HLA-G) and only one polymorphic classical class I molecule, HLA-C. CD94/NKG2 heterodimer receptors, expressed by uNK cells, bind to non-classical HLA-E molecules. Three receptors to which HLA-G binds have been identified, including killer cell immunoglobulin-like receptor (KIR) *KIR2DL4*. Finally, HLA-C is the ligand for KIR receptors (highly polymorphic as well). In turn, KIR receptors are classified according to how many Ig-like extracellular domains they present (KIR2D if they have two, or KIR3D if they have three). In addition, whether the cytoplasmic tail is long (KIR2DL and KIR3DL) or short (KIR2DS and KIR3DS) is also considered. Adapted from Moffett and Colucci (2015). Created with BioRender.com.

(Moffett *et al.*, 2016). The KIR B haplotype consists of any other combination of the KIR loci (Rajalingam *et al.*, 2008).

A woman carries alleles for multiple KIR genotypes, often from both inhibitory and activating categories. However, linkage disequilibrium causes some genes to not segregate independently, tending to migrate together (Parham, 2004). This is why the KIR genotype profile of each person is broadly classified into two haplotypes: KIR A and KIR B. In any pregnancy, the maternal KIR genotype could be AA (no activating KIR), AB or BB (only activating KIR) (Uhrberg *et al.*, 1997). uNK cells, in contrast with pbNK cells, express KIR receptors that are specific for HLA-C. This indicates that the recognition of HLA-C by NK cells is much more pronounced in the uterus (Hiby *et al.*, 1997; Verma *et al.*, 1997). Furthermore, in the population of $CD56^{+}$ pbNK cells with a lower cytotoxic capacity, the expression of certain KIRs is more diminished or even absent (Jacobs *et al.*, 2001). This has given rise to the possibility that uNK cells ($CD56^{+}$ and of low cytotoxicity) express these KIR receptors induced by the uterine environment (Moffett-King, 2002).

The MHC constitutes a fundamental pillar of the vertebrate immune system. Its main function is to regulate adaptive immunity against pathogens mediated by T cells (Fernandez *et al.*, 1999). During pregnancy, the maternal immune system undergoes changes that lead to tolerance of the fetus (Fernandez *et al.*, 1999). The most fundamental expressed genes in the trophoblast are related to the MHC and MHC-like since they are ligands of the uNK cells. Although the fetus expresses all the MHC genes (Loke and King, 1995), it is the trophoblast that comes into direct contact with the maternal immune system. The EVT expresses some MHC antigens, but it does not express MHC class II molecules or the two main classical MHC class I antigens, HLA-A or HLA-B. Four MHC class I antigens (HLA-C, HLA-G, HLA-E and HLA-F) have been detected in the trophoblast (Hiby *et al.*, 1999; King, 2000; Ishitani *et al.*, 2003; Trundley and Moffett, 2004; Shobu *et al.*, 2006; Hackmon *et al.*, 2017).

HLA antigens are glycoproteins that belong to the immunoglobulin superfamily present on most cell surfaces in the body. HLA antigens present in specialized immune cells allow the peptides of foreign substances (viruses, bacteria, etc.) to be exposed to the effector cells of the immune system. Therefore, their main function is to serve as recognition molecules during the initiation of an immune response (Scaradavou, 2013). EVTs express the classical class I HLA-C molecules and the non-classical class I HLA-E, HLA-F and HLA-G molecules, while HLA-A and HLA-B class II antigens are absent (King, 2000; Apps *et al.*, 2009; Hackmon *et al.*, 2017). HLA-E, HLA-F and HLA-G are non-classical genes located in the human MHC on chromosome 6 (Hviid, 2006), discovered in the 1980s (Geraghty *et al.*, 1987, 1990; Koller *et al.*, 1988). Since then, HLA-G has been the most extensively studied, including determining its functions and expression in the trophoblast (Kovats *et al.*, 1990; Ishitani and Geraghty, 1992; Ishitani *et al.*, 2003). HLA-C is highly polymorphic (6,067 alleles) (Antony Nolan Research Institute, 2020) and the paternal allele is expressed on the trophoblast cell surface. Thus, in terms of recognition in the context of placentation, HLA-C in the trophoblast is the most relevant MHC class I molecule. The HLA-C genotypes expressed by EVT cells are generally organized into two groups defined by a dimorphism at position 80 of the α1 domain, according to the amino acid, HLA-C1: asparagine; HLA-C2: lysine (Mandelboim *et al.*, 1996). Group C1 allotypes (antibody chain alleles) are ligands for inhibitory receptors *KIR2DL2* (haplotype B) and *KIR2DL3* (haplotype A). Group

C2 allotypes show a much stronger inhibitory effect when binding to *KIR2DL1* inhibitory receptors (haplotype A) than group C1 allotypes binding with their corresponding inhibitory receptors (listed above). HLA-C2 also binds to the activating receptor *KIR2DS1* (haplotype B) (Winter *et al.*, 1998).

HLA-G is a non-classical human leukocyte antigen located mainly at the maternal–foetal interface, suggesting its involvement in the establishment and maintenance of pregnancy (Ellis *et al.*, 1990; Kovats *et al.*, 1990). HLA-G expression is quite restricted, and it has a low level of polymorphisms (80 alleles) (Antony Nolan Research Institute, 2020). Three receptors that appear to interact with HLA-G have been identified: ILT2 (Colonna *et al.*, 1997; Allan *et al.*, 1999), ILT4 (Allan *et al.*, 1999) and *KIR2DL4* (Ponte *et al.*, 1999; Rajagopalan and Long, 1999). Specifically, *KIR2DL4* and ILT2 receptors are expressed by NK cells.

HLA-G is widely expressed in the placenta and has been associated with cytokine secretion, which, in turn, could influence placental development, invasion of the trophoblast, transformation and vascular remodeling (Roth and Fisher, 1999; Li *et al.*, 2001; Ingman and Robertson, 2002). Membrane-bound HLA-G seems to promote specific functions of the uNK cells related to implantation and decidualization (van der Meer *et al.*, 2004), favouring uNK cell proliferation and interferon-γ (IFN-γ) and vascular endothelial growth factor (VEGF) production (van der Meer *et al.*, 2004). Foetal and placental weights were analysed in patients who experienced severe pre-eclampsia during pregnancy and compared with the same measures in pregnancies that developed without this complication (Hviid, 2004). Patients with a homozygous HLA-G genotype, based on the presence of a 14-base pair (bp) sequence polymorphism, were found to have babies with significantly higher birthweights (Hviid, 2004). Based on these results, it has been suggested that certain clinical parameters, such as placental and foetal weight, could be influenced by the HLA-G polymorphism (Hviid, 2006). Although in different trimesters of pregnancy, several studies have found levels of serum soluble HLA-G (sHLA-G) to be significantly lower in women with pre-eclampsia compared to healthy pregnancies (Yie *et al.*, 2005; Hackmon *et al.*, 2007; Darmochwal-Kolarz *et al.*, 2012; Rokhafrooz *et al.*, 2018). Therefore, a low concentration of sHLA-G has been associated with an increased risk of developing pre-eclampsia (Steinborn *et al.*, 2007; Alecsandru and Garcia-Velasco, 2020).

On the other hand, the presence of HLA-E transcripts has been described in EVT cells in the placenta and in choriocarcinoma cell lines (Wei and Orr, 1990; Ulbrecht *et al.*, 1992; Guillaudeux *et al.*, 1995). The surface expression of HLA-E is regulated by the binding of peptides derived from sequences of other MHC class I molecules (Braud *et al.*, 1997, 1998b). CD94/NKG2 heterodimer receptors, expressed by uNK cells (Verma *et al.*, 1997), bind to non-classical HLA-E molecules (Braud *et al.*, 1998a). Depending on the origin of the HLA-E-linked peptides that interact with CD94/NKG2C, activation or inhibition of NK cells has been demonstrated (Lee *et al.*, 1998; Llano *et al.*, 1998). HLA-E expression increases considerably from the 20th week of gestation, which coincides with the time of rapid increase in foetal weight. This suggests that its functions may be related to the release of cytokines by uNK cells, favouring and ensuring sufficient nutritional supply for the growth of the fetus (Shobu *et al.*, 2006).

HLA-F is expressed on the surfaces of placental EVTs in the last period of gestation (Ishitani *et al.*, 2003), and in the cytoplasm of EVT cells during early pregnancy (Shobu *et al.*, 2006). The exact functions of this molecule have not yet been elucidated. Although HLA-F tetramers were reported to bind to NK receptors ILT2 and ILT4 (Lepin *et al.*, 2000), there has been no evidence to verify if and how HLA-F interacts with NK cells.

KIR/HLA-C combinations: impact on reproduction

Given that each pregnancy involves different combinations of paternally derived foetal HLA-C and maternal KIR, it was suggested that some of these combinations were not optimal for proper implantation, which could lead to disorders such as pre-eclampsia or RM (Moffett-King, 2002). Therefore, the wide variety of foetal KIR and HLA-C maternal ligands could mean that certain KIR/HLA-C combinations would favour greater reproductive success than others, primarily due to signals received by uNK cells (Hiby *et al.*, 2010a).

Hiby *et al.* (2004, 2008) found a significant increase in the KIR AA genotype frequency in women with RM and pre-eclampsia compared to a control population. When a woman is homozygous for the KIR AA genotype, the fetus has more HLA-C2 genes than the mother and the additional foetal HLA-C2 alleles are of 'foreign' origin (inherited from the father or from donated oocytes) and there is not an adequate arterial transformation, which translates into increased risk of RM, pre-eclampsia or FGR (Alecsandru *et al.*, 2014, 2020; Moffett *et al.*, 2016). These data suggest that HLA-C2 may have a deleterious allogeneic effect (Hiby *et al.*, 2004, 2008; Hiby *et al.*, 2010a,b). *KIR2DL1* (inhibitory KIR for C2 epitope) has been proposed to be the main cause of this negative effect since, when the KIR AA genotype is present, strong uNK inhibition occurs, mediated by the binding of *KIR2DL1* to the trophoblast HLA-C2 (Moffett *et al.*, 2016). When a woman presents two KIR A haplotypes (KIR AA), only receptors that exert a strong inhibitory signal on the uNK cells are coded (inhibitory KIR genes). On the other hand, women who express the KIR B haplotype (which contains activator genes) in which the C2 receptor activator (*KIR2DS1*) is present, are significantly protected against these pregnancy disorders (Hiby *et al.*, 2010a). Thus, strong inhibition of uNK cells after trophoblast binding is detrimental to placentation, triggering arterial transformation disorders, while activation of uNK cells counteracts this effect (Hiby *et al.*, 2014).

Couples with RM undergoing IVF were analysed genetically and one of the KIR genes of haplotype B was shown to be protective against this pathology (*KIR2DS1*). The absence of *KIR2DS1* in women with RM was found to be significant (Hiby *et al.*, 2008). Thus, *KIR2DS1* could be activating the receptor for C2 groups, compensating for the inhibitory effect generated by the C2-*KIR2DL1* interaction (Stewart *et al.*, 2005). Furthermore, when this binding of *KIR2DL1* to C2 occurs, the production of angiogenic factors and cytokines by uNK cells is decreased (Hiby *et al.*, 2008). Although a combination of maternal *KIR2DS1* with foetal HLA-C2 inherited from the father protects against poor placentation, it has also been associated with increased birthweight, increasing the risk of a macrosomic baby (Hiby *et al.*, 2014). This highlights the importance of balanced activation of these cells as an essential requirement for successful implantation.

The transfer of more than one embryo at a time, although much less frequent than previously, is still performed in some circumstances.

Donor oocytes are used much more frequently. These situations translate into a higher number of non-self-antigens (HLA-C) presented to the mother's uNK cells KIR receptors compared to what happens in natural pregnancies. During IVF treatments, embryo transfers can even be performed quite regularly in patients with recurrent implantation failure (RIF) (Das and Holzer, 2012). Therefore, the presentation of these HLA-C antigens occurs much more frequently than in a natural pregnancy (Alecsandru and Garciá-Velasco, 2015), associating oocyte donation with an increased risk of morbidity during pregnancy (Pecks *et al.*, 2011).

Notably, significantly decreased live birth rates were observed in KIR AA patients whose embryos had an increased expression of HLA-C2 (Alecsandru *et al.*, 2020). This difference was not observed in KIR AB or KIR BB patients when HLA-C2 expression was also increased (Alecsandru *et al.*, 2020). In fact, there is great concern especially for those women who receive donated oocytes, as the risk of pre-eclampsia has been reported to be considerably increased, even in young oocyte recipients (Levron *et al.*, 2014). A 25% risk of pre-eclampsia has been reported in oocyte donations versus a 10% risk in other women who undergo ART (Moffett *et al.*, 2016). Curiously, the KIR AA genotype and HLA-C2 frequencies vary between populations and appear to be inversely related. This inverse relation supports the hypothesis that reproduction has exerted selective pressure on the KIR and HLA-C variety (Hiby *et al.*, 2004). Certain combinations of maternal KIR and foetal HLA-C genes seem to influence the risk of developing pre-eclampsia and reproductive success. Upon consulting and comparing data from different populations, an inverse relation between the population prevalence of HLA-C2 and AA genotypes was observed: that is, the higher the frequency of C2, the lower the frequency of the KIR AA genotype (Hiby *et al.*, 2004).

Reproduction exerts selective pressure for KIR and HLA-C diversity, so it is likely that the KIR and HLA regions have evolved rapidly to prevent risky pregnancy combinations, as they are located on separate chromosomes and segregate independently. In the Japanese population, the frequency of the KIR A haplotype is high, while the frequency of HLA alleles that carry the C2 epitope is low compared to the frequencies in other populations (Hiby *et al.*, 2004). The incidence of the KIR AA genotype in Japanese women is 60%. However, the frequency of HLA-C alleles with the C2 epitope is more than three times higher in the European population (32%) than in the Japanese population (9%) (Moffett *et al.*, 2016). Saito *et al.* found that pre-eclampsia rates were similar between couples with fully Japanese ancestry and couples of mixed ancestries (Japanese women and Caucasian men), indicating that the HLA-C/KIR genotype was not associated with pre-eclampsia (Saito *et al.*, 2006). However, some concerns about the sample size of the study have been recently raised (Moffett *et al.*, 2016).

uNK cells and angiogenesis: a balanced relationship

Invasion of the trophoblast during placentation is one of the most critical moments in the entire pregnancy process. Although the extent to which uNK cells could regulate this process has not yet been determined *in-vivo, in-vitro* studies have demonstrated that these cells can induce trophoblast invasion (Fraser *et al.*, 2012). As will be seen in the next section, uNK cells secrete a series of growth factors that seem to be involved in decidual-associated remodeling and, therefore, in the arterial remodeling that this entails (Lash, 2006).

Many changes occur during remodeling of the spiral arteries, including increased permeability and dilation of vessels, transient loss of endothelial cells, separation of VSMC, endovascular invasion of EVT, and presence of intramural EVT (Lash *et al.*, 2010c). This vascular remodeling process is suggested to be regulated by paracrine signaling of factors produced by uNK cells (Robson *et al.*, 2019). An *in-vitro* model evaluating possible angiogenic factors derived from uNK cells that could be involved in this dedifferentiation of VSMCs (Lash, 2006; Lash *et al.*, 2010a) identified Ang-1 and Ang-2 (Robson *et al.*, 2019). However, as will be seen in the next section, several angiogenic factors produced by uNK cells have been associated with endometrial angiogenesis (Trundley and Moffett, 2004; Lash, 2006). In fact, differences have been found in the expression levels of these factors between fertile women, and women with RM and RIF (Chen *et al.*, 2016b, 2017a).

The study of angiogenesis and its relationship to uNK cells reveals two opposite trends associated with increased risk of pregnancy disorders, the underinvasion or the overinvasion of the trophoblast in the decidua. On one hand is deficient arterial transformation with low production of angiogenic factors (VEGF-A/C, placental growth factor (PLGF), TGF-β1 and angiopoeitin) and insufficient invasion of the trophoblast (Trundley and Moffett, 2004; Hiby *et al.*, 2008). On the other hand, an opposite effect is increased production of these angiogenic factors that translates into greater peri-implantation blood flow and thus an increase in the oxidative process (Quenby *et al.*, 2009; Chen *et al.*, 2017a).

Several studies indicated a relation between impaired vascular development and miscarriage (De Agustín *et al.*, 1971; Ball *et al.*, 2006; Chen *et al.*, 2016a). Angiogenesis and an adequate blood supply are critical steps in pregnancy, especially in its earliest stages. Angiogenesis and vasoconstriction-related genes may be associated with RM. A meta-analysis of genes related to angiogenesis and vasoconstriction evaluated a possible association of these genes with idiopathic RM. VEGF (2154 G.A), p53 (codon72) and endothelial nitric oxide synthase (Glu298Asp) polymorphisms showed significant associations with idiopathic RM (Su *et al.*, 2013). Ball *et al.* (2006) studied the relation between trophoblast invasion and arterial transformation with late miscarriage. Invasion of the trophoblast in the decidua and myometrium was reduced in women who had suffered a late miscarriage, and the spiral arteries in late miscarriage showed reduced endovascular change. These results suggest that inadequate transformation of the spiral arteries and poor invasion of the trophoblast could not only be associated with disorders such as preeclampsia and FGR but also with late miscarriage (Ball *et al.*, 2006).

As previously indicated, certain KIR/HLA-C combinations have been associated with increased risk of pregnancy disorders such as preeclampsia or RM (Alecsandru *et al.*, 2014, 2020; Moffett *et al.*, 2016). These results were linked to a strong inhibitory effect of uNK cells, decreasing production of angiogenic factors and cytokines (Hiby *et al.*, 2008) to yield deficient placentation (Hiby *et al.*, 2010a,b; Faridi and Agrawal, 2011). This process translates into insufficient invasion of the decidua by the EVT, one of the main defects that occur in

disorders such as pre-eclampsia, FGR, or RM (Alecsandru and García-Velasco, 2015).

However, numerous studies have reported opposing results. Increased numbers of uNK cells have also been detected in the peri-implantation endometrium of women with RM (Clifford et al., 1999; Tuckerman et al., 2007). uNK cells isolated from women with RM were found to produce more angiogenic factors compared to fertile women (Chen et al., 2016b). It has also been proposed that a high number of uNK cells increases angiogenesis and thus peri-implantation blood flow (Quenby et al., 2009). Together, this information suggests that angiogenesis in the endometrium is increased in women with RM (Chen et al., 2017a). In fact, it has been observed that women with RM have an increased number and volume of micro-blood vessels in the peri-implantation endometrium (Chen et al., 2016a). On the other hand, this increase in angiogenesis has also been related to higher levels of progesterone. Women with elevated progesterone levels on the same day and the day after hCG administration have significantly upregulated genes of the killer cell lectin-like receptors (KLRs) family (Liu et al., 2017). These genes are involved in cytotoxicity, with production of cytokines and angiogenic factors. Another aspect is an 'over-activation' that KIRBB receptors (specifically *KIR2DS1*) could generate on uNK cells, as the frequency of *KIR2DS1* is significantly higher in pregnancies with a high birthweight (Hiby et al., 2014).

Early vascular remodeling causes an increase in blood flow in the vascular bed, a decrease in the speed of blood entering the placental region, greater blood supply and greater supply of oxygen to the maternal–foetal interface (Thaler et al., 1990; Burton et al., 2009; Hofmann et al., 2014). Variation in the oxygen supply would cause an alteration in oxygen tension within the utero-placental environment. Invasion of the trophoblast is favoured in hypoxic conditions (Na et al., 2012), therefore, changes in oxygen tension have consequences in trophoblast invasion. From here, it is suggested that uNK cells can indirectly regulate trophoblast invasion by regulating oxygen tension at the maternal–foetal interface (Chakraborty et al., 2011). During the first trimester of pregnancy, the placenta is highly sensitive to the effect of oxygen as a consequence of low concentrations of antioxidant enzymes (Quenby et al., 2009). In abnormal early pregnancies, placental oxidative stress levels have been reported to be higher than normal (Jauniaux et al., 2003). Also, a correlation exists between the number of uNK cells present in the endometrium and increased oxidative stress and trophoblast degeneration (Quenby et al., 2009). Therefore, it seems that a relatively hypoxic environment promotes correct embryo implantation and early development of the placenta (Yedwab et al., 1976; Rodesch et al., 1992), minimizing the detrimental effect of reactive oxygen species (Yang et al., 1998). This is closely related to endometrial angiogenesis, which, as will be seen in the next section, can be favoured by uNK cells.

Taking all this information into account, an increase in endometrial angiogenesis activity induced by an increase in uNK cells and their production of cytokines and angiogenic factors could be detrimental to embryo implantation owing to increased arrival of oxygen, thus increasing oxidative stress (Chen et al., 2017a). This is currently being studied as a possible cause of RM (Chen et al., 2017a).

uNK cytokine production: open research

uNK cells are an important source of growth factors and cytokines (Li et al., 2001; Hanna et al., 2006; Lash, 2006). For this reason, two main functions have been attributed to them—promoting vascular remodeling and regulating trophoblast invasion (Hanna et al., 2006; Lash et al., 2010b).

During invasion of the trophoblast and remodeling of the spiral arteries, numerous growth factors, cytokines, and hormones are secreted from the trophoblast and decidua to regulate these processes (Kharfi et al., 2003; Lala and Chakraborty, 2003; Bischof and Irminger-Finger, 2005). uNK cells have been characterized by their role in the production of different cytokines as well as growth factors involved in the regulation of trophoblast invasion (Hanna et al., 2006; Lash et al., 2010c) and in the initial stages of spiral artery remodeling (Lash, 2006; Smith et al., 2009; Lash et al., 2010c). uNK cytokine profiling has been investigated during the early stages of pregnancy: granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), tumour necrosis factor-α (TNF-α), IFN-γ and leukaemia inhibitory factor (LIF) were the main molecules produced by these cells (Saito et al., 1993; Jokhi et al., 1994).

uNK cells are also a major source of VEGF-C, Ang1, Ang2 and TGF-β1 (Lash, 2006), suggesting that uNK cells may play an important role in endometrial angiogenesis. uNK cells secrete other pleiotropic cytokines such as IL-1β, IL-6, IL-8 and IFN-γ, which increase with gestational age (Lash et al., 2010c). Indeed, VEGF-A, PLGF and angiopoietin (Trundley and Moffett, 2004) are among the cytokines and angiogenic growth factors produced by uNK cells that could direct angiogenesis during embryo implantation (Chen et al., 2017a). It is unknown whether there are differences in the expression levels of growth and angiogenic factors produced by uNK cells during the menstrual cycle and early pregnancy. The levels of these proteins secreted by uNK cells vary throughout pregnancy (Lash, 2006). Ang2, Ang1 and VEGF-C proteins are detected at lower levels in weeks 12–14 of pregnancy than in weeks 8–10 of pregnancy (Lash, 2006; Lash et al., 2010c).

In fact, co-culture of uNK cells with either EVT or cytotrophoblast suggests that the interaction between these cells could mediate decreased secretion of both growth factors and cytokines (Lash et al., 2011). Cytokine secretion decreases only when both cell types are in direct contact (IL-6 and IL-8) (Lash et al., 2011). These interactions could play a role in controlling the invasiveness of EVT. Differences in cytokine and growth factor secretion (IL-6, TGFβ1 or inhibin-A/B among others) have been observed when comparing term pregnancy EVT and chorionic EVT with first trimester EVT (HLA-G + in all three cases) (Papuchova et al., 2020). Furthermore, significant changes were also seen in their phenotypes and gene expression profiles, suggesting that first trimester and term pregnancy EVTs employ different mechanisms to interact with immune cells present in the decidua (Papuchova et al., 2020).

Ang1 and Ang2 exert their effect through the Tie2 receptor. Ang1 has proangiogenic properties, while Ang2 binds competitively to the Tie2 receptor, inhibiting its effect (Maisonpierre et al., 1997). Furthermore, although trophoblastic interstitial cells express some

receptors for these cytokines (GM-CSF, IFN-γ, CSF1), the problem appears when studying the functional role of these cytokines. Isolating EVT cells for *in-vitro* studies is not an easy task, nor is the use of cell lines to simulate what happens *in vivo* with these EVTs (King *et al.*, 2000; Moffett-King, 2002). Nonetheless, Xiong *et al.* (2013) evaluated how inhibitory *KIR2DL1* and activating *KIR2DS1* appear to confer protection or increase the risk of certain reproductive disorders: they demonstrated that activation of *KIR2DS1* by HLA-C2 from uNK cells promoted the production of cytokines and other factors, including GM-CSF. In turn, GM-CSF promoted the migration of the primary trophoblast and choriocarcinoma cells (JEG3) *in vitro* (Xiong *et al.*, 2013).

Immune therapies: what are we fighting against?

Although the trophoblast cells invade the mother's uterine tissue just as cancer cells do, there is no rejection response from the NK cells. In fact, uNK cells do not generate a cytotoxic rejection response, but instead positively interact with the trophoblast during implantation and early stages of pregnancy (Colucci, 2017). The role of the immune system in cases of RM and RIF and its implications for assisted reproduction remain controversial. However, much of this controversy is related to the fact that, until now, many of the studies carried out on this topic have focused on the search for markers in peripheral blood rather than directly on uNK cells (Alecsandru and Garciá-Velasco, 2015; Alecsandru and García-Velasco, 2017). Based on a controversial concept that immunomodulatory drugs could be an effective therapy for reproductive disorders such as RM or RIF, different treatments, including steroids, IVIG, and intralipids (see below), have been used (Table I) with discordant results. These types of immune-modulating therapies include paternal leukocyte immunization, progesterone, aspirin, steroids (Wong *et al.*, 2014), TNFα inhibitors, IVIG (Christiansen *et al.*, 2002; Winger *et al.*, 2011; Moraru *et al.*, 2012), intralipid (Shreeve and Sadek, 2012), prednisolone or dexamethasone and G-CSF (Ubaldi *et al.*, 2002; Motteram *et al.*, 2015). However, the effectiveness of these treatments is mostly not scientifically accepted, so it is still unknown if they really affect only uNK cells of the immune system and exactly what type of effect is generated (Moffett and Shreeve, 2015).

Lymphocyte immunotherapy (LIT) began to be used in the 1980s to treat infertility and miscarriage. Infusions of paternal leukocytes were administered to women with the intention to generate a beneficial immune response to paternal antigens (Mowbray *et al.*, 1985). To accomplish this, women were injected with purified lymphocytes from the blood of their male partners (Mowbray *et al.*, 1985). The scientific basis of this technique was always quite doubtful because, although the maternal immune system is activated against the paternal antigens expressed by the fetus, the immune response that is generated to EVTs in the uterus must be considered separately (Moffett and Shreeve, 2015). In 2002, the US Food and Drug Administration (FDA) banned this treatment (Wong *et al.*, 2014). In the 1990s, a new test was launched to measure pbNK cells (Chao *et al.*, 1995), and women who had a high number of these cells were offered therapies to prevent NK cells from attacking the embryo (Ruiz *et al.*, 1996). Apart from immunization with paternal cells, other approaches have also been evaluated, such as those using leukocytes from third-party donors or trophoblast membranes. None of them improve the live birth rate after application (Wong *et al.*, 2014).

Another immune therapy consists of the supply of a fat emulsion composed of soybean oil, glycerine and egg phospholipids, known as intralipid. It is generally used as a component of parenteral nutrition in patients who cannot tolerate an oral diet (Driver *et al.*, 1989). Although it is not entirely clear how intralipid exerts its immunomodulatory effect, the ability of soybean oil to inhibit pro-inflammatory mediators is thought to be involved (Granato *et al.*, 2000). In 1985, it was verified that incorporation of intravenous intralipid to the total parenteral nutrition regimen does not modify immune function (Ota *et al.*, 1985). However, different studies have sent conflicting messages.

Few studies have evaluated the effect of intralipid in reproduction, and promising new treatments such as this can be a double-edged sword (Shreeve and Sadek, 2012). Intralipid is an inexpensive and interesting option because of its possible application in reproductive clinics, but its efficacy has not been tested, so its application in patients is risky (Shreeve and Sadek, 2012). In fact, a prospective cohort study in women aged 40–42 years assessing the efficacy of intralipid therapy had to be interrupted before the established time because the preliminary data did not show any live births in the treatment group, while the live birth rate in the control group was 30% (without treatment) (Check and Check, 2016).

IVIG preparations are composed of a pool of plasma obtained from blood samples from different human donors (Chen *et al.*, 2000; Constantine *et al.*, 2007). To verify the effectiveness of IVIG treatment in preventing embryo implantation failure, the reproductive results of patients with previous miscarriages and implantation failures were analysed. Although the implantation rate was significantly higher in treated patients, the pregnancy rate was not increased (Placido *et al.*, 1994). Furthermore, a meta-analysis carried out by Polanski compared the reproductive results of three different studies of IVIG administration and the use of oral prednisolone. Although beneficial clinical results after the application of immunotherapy were seen in all of the included studies, this meta-analysis did not support 'the use of prednisolone, IVIG, or any other adjuvant treatment in women undergoing ART who are found to have elevated absolute numbers or activity of NK cells' (Polanski *et al.*, 2014). This conclusion was established after verifying the low statistical power of the individual studies (Alhalabi *et al.*, 2011; Winger *et al.*, 2011; Moraru *et al.*, 2012) as well as the absence of good-quality evidence. All the studies included in this meta-analysis used pbNK cell levels as a diagnostic test. Therefore, the lack of scientific support for the predictive value of these data and the scarcity of available data to support these treatments highlights the need for the scientific community to reach an agreement on the most appropriate methodology to follow and the NK levels to be taken as reference. Once again, the validity of these tests that continue to be offered to couples with ART is questioned.

Some studies have shown positive reproductive results after corticosteroid administration (De Fried *et al.*, 1993; Dan *et al.*, 2015). A meta-analysis showed that prednisolone therapy improved pregnancy outcomes in patients with RM, but there were no significant differences in pregnancy outcomes in ICSI cycle patients (Dan *et al.*, 2015). However, some studies show no beneficial effects of corticosteroids on implantation rates in patients receiving IVF or ICSI

Table I Summary of immune therapy studies in patients undergoing ART.

Immune therapy	Type of study	Patients	General description (treatment/duration/objectives)	Results/Conclusions	Publication
	Meta-analysis	Nine randomised trials (seven double-blinded) were evaluated. 430 patients were included in analysis 1 and 449 in analysis 2.	Data from all patients experiencing RM who had participated in clinical trials of allogeneic leukocyte immunization worldwide were studied by two independent analyses (with different definitions and statistical methods).	In both analyses 1 and 2, the live birth rates (LBR) were significantly higher after the application of immunotherapy ($P = 0.025$ and $P = 0.024$, respectively)	Coulam *et al.* (1994)
	Meta-analysis	Four randomised-controlled trials and 19 case-series reports.	A meta-analysis was performed to evaluate the efficacy of immune therapies to treat RM.	Recommended abandoning this treatment.	Fraser *et al.* (1994)
	Meta-analysis and logistic regression	A total of 285 patients.	Data from randomised-controlled trials in eight centres were analysed to assess the effectiveness of allogeneic leukocyte immunization in RM patients in terms of LBR.	The LBR was significantly higher in the group that received the treatment compared to the group that did not ($P = 0.008$).	Daya and Gunby (1994)
	Randomised multicentre controlled trial	A total of 44 women entered the study. Patients were randomly allocated to immunotherapy or expectant management.	The efficacy of immunotherapy with paternal leukocytes to treat unexplained RM was evaluated.	No statistically significant differences were observed in reproductive outcomes between groups.	Illeni *et al.* (1994)
Lymphocyte immunotherapy	Placebo-controlled randomised trial	46 women with three or more consecutive first trimester miscarriages.	Evaluated whether administration of paternal mononuclear cells in women with RM improved reproductive outcomes.	No significant differences were observed between groups.	Cauchi *et al.* (1991)
	Placebo-controlled randomised trial	99 women with three or more consecutive spontaneous abortions.	Women received their own lymphocytes (control group), those of their husbands or were immunized with lymphocytes from third parties.	No significant improvements in LBR were obtained when comparing women immunized with lymphocytes from their husbands or with lymphocytes from third parties with those in the placebo group.	Ho *et al.* (1991)
	Double-blind, placebo-controlled, randomised trial	124 women with RM.	Patients received six identical immunizations every four weeks.	Women who received immunization with paternal lymphocytes showed significantly increased pregnancy success ($P < 0.001$).	Pandey and Agrawal (2004)
	Double-blind, multicentre, randomised clinical trial	179 women with RM (with three or more unexplained miscarriages). 89 patients in the treatment group and 90 in the control group.	Investigated whether paternal mononuclear cell immunization improves the rate of successful pregnancies.	The number of live births between the immunized group and the control group were not significantly different.	Ober *et al.* (1999)
	Randomised and non-randomised trials	489 patients with three or more consecutive pregnancy losses and patients from an international register (other centres) (1431).	The effect of immunopotentiation in patients with five or more miscarriages was analysed.	In primary and tertiary abortion patients who were immunized, the LBR was significantly increased ($P = 0.0015$ and $P = 0.002$, respectively).	Carp *et al.* (1997)
	Double-blind, prospective, placebo-controlled trial	66 women with RM.	Investigated whether immunization with third party leukocytes improves the reproductive outcomes of women with unexplained RM.	The success rate (pregnancies with live births) was not significantly different between groups. Serious adverse effects were recorded in women and newborns.	Christiansen *et al.* (1994)
	Double-blind, placebo-controlled trial	47 women with unexplained RM (three or more consecutive spontaneous miscarriages).	Evaluated whether immunization with paternal lymphocytes improves reproductive outcomes.	There were no statistically significant differences in LBR between groups.	Gatenby *et al.* (1993)
	Double blind trial	97 couples (at least three miscarriages with the same partner).	One group was injected with the husband's lymphocytes and another with their own lymphocytes.	The pregnancy outcomes were significantly better in the immunised group than the control ($P = 0.01$).	Mowbray *et al.* (1985)

Continued

Table I Continued

Immune therapy	Type of study	Patients	General description (treatment/duration/objectives)	Results/Conclusions	Publication
Lymphocyte immunotherapy	Observational	50 women with three or more consecutive unexplained first-trimester miscarriages.	Use of intravenous infusion with purified trophoblast membrane preparations as immunotherapy.	A total of 16/21 (76%) patients who subsequently had pregnancies achieved a live birth or were currently pregnant at greater than 28 weeks gestation.	Johnson *et al.* (1988)
Corticosteroids	Meta-analysis	Five studies were selected that met the inclusion criteria.	This meta-analysis evaluated the efficiency of prednisolone administration on unexplained RM and in women undergoing assisted reproductive technology.	Prednisolone therapy improved pregnancy outcomes in women with idiopathic RM, while its efficacy in women undergoing intracytoplasmic sperm injection (ICSI) was not significant.	Dan *et al.* (2015)
	Prospective non-randomised study	56 patients with tubal factor infertility (FIV patients). Patients were divided into one group with treatment (60 mg of methylprednisone for 4 days) and another group without treatment.	The effect of corticosteroids on the PrR and IR of patients undergoing IVF and embryo transfer were evaluated.	The group that received immunosuppressive treatment achieved a statistically significant increase ($P < 0.01$) in pregnancy and take home baby rates.	De Fried *et al.* (1993)
	Prospective randomised trial	175 infertile women.	The efficacy of immunosuppression with corticosteroids to improve PrR and IR was evaluated. Patients received multiple doses of 16β-methylprednisolone (0.16 or 60 mg/day) for 4 days.	PR and IR did not show statistically significant differences between groups.	Lee *et al.* (1994)
	Randomised, prospective, double-blind, placebo-controlled trial	267 IVF patients. Patients having micromanipulation were excluded.	The effect of low-dose glucocorticoid treatment on the PrR was evaluated. Patients were assigned 16 mg oral 6-α-methylprednisolone for 4 evenings. They were administered 250 mg oral tetracycline four times per day (4 days).	The IR and clinical PrR between placebo and glucocorticoid groups showed no statistically significant differences.	Moffitt *et al.* (1995)
	Randomised, prospective study	313 patients were divided into one group with treatment (10 mg/day) and another without treatment.	The effect of low-dose prednisolone on PrR and IR in ICSI patients was evaluated.	No significant effect on PrR or IR was found.	Ubaldi *et al.* (2002)
	Randomised, prospective, placebo-controlled study	200 patients undergoing ICSI.	The effect of aspirin together with prednisolone was evaluated on IR and PrR were evaluated.	There were no statistically significant differences in PR between groups.	Duvan *et al.* (2006)
	Prospective randomised study	97 patients who received acetylsalicylic acid (ASA) + prednisolone and another 298 patients who did not receive any medication were selected. All of them had a good pregnancy prognosis.	Evaluated if therapy with ASA and prednisolone improved the reproductive results of IVF.	PrR and IR did not show statistically significant differences between groups.	Revelli *et al.* (2008)
	Randomised placebo-controlled study	170 women with idiopathic RM.	LBR and pregnancy outcomes were compared between two groups of women with idiopathic RM. Some received enoxaparin, others received a combination therapy of prednisone, aspirin and progesterone and others a placebo.	The LBR was significantly higher in both the enoxaparin and combination therapy groups than the placebo group ($P < 0.05$).	Fawzy *et al.* (2008)
	Randomised-controlled prospective study	112 women undergoing ICSI treatment who had more than 1% of their total lymphocytes consisting of NK cells expressing activation marker $CD69^{+}$.	The effect of prednisolone was evaluated and pregnancy outcome was assessed.	The clinical PrR was significantly higher ($P < 0.05$) in the prednisolone group compared to the control group.	Alhalabi *et al.* (2011)

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Table I Continued

Immune therapy	Type of study	Patients	General description (treatment/duration/objectives)	Results/Conclusions	Publication
Corticosteroids	Double-blind, placebo, randomised-control trial	160 patients with RM.	The efficacy of prednisolone combined with heparin was evaluated in contrast to the administration of heparin alone in patients with unexplained RM (two groups were generated, one received oral prednisolone and low dose aspirin and heparin and the second group received placebo and low dose aspirin and heparin).	The percentage of successful pregnancies (pregnancy over 20 weeks) was significantly increased ($P < 0.05$) in the prednisolone group.	Gomaa *et al.* (2014)
	Cohort study	115 women with RIF undergoing IVF/ICSI treatment.	Evaluated whether the administration of low molecular weight heparin (LMWH) and prednisolone improve clinical outcomes in patients with RIF undergoing IVF/ICSI treatment.	Pregnancy, miscarriage and LBR did not show statistically significant differences between groups.	Siristatidis *et al.* (2018)
	Matched-pair study	210 patients with idiopathic RM.	Evaluated whether administration of a combined treatment of prednisone, aspirin, folate and progesterone has a beneficial effect on the reproductive results of women with idiopathic RM.	The LBR of the treatment group was significantly higher compared to the control group ($P = 0.04$).	Tempfer *et al.* (2006)
	Matched case-control study	Cases (n = 485) and controls (n = 485) were matched according to physical and clinical parameters.	LBR was evaluated after the transfer of fresh and frozen embryos in both IVF and ICSI cycles.	No significant differences were observed when fresh embryos were transferred. With frozen embryos, the LBR was lower in the treatment group.	Motteram *et al.* (2015)
Intravenous immunoglobulin (IVIG)	Meta-analysis	Four randomised, double-blind trials comparing IVIG with placebo for treatment of RM were included in this meta-analysis.	The effectiveness of IVIG as a treatment for RM was evaluated.	The effect of IVIG treatment was not statistically significant ($P = 0.16$). After excluding pregnancy losses that were caused by factors that could not be prevented by treatment or were unrelated to placebo use, the effect was significant ($P = 0.049$).	Daya *et al.* (1998)
	Meta-analysis	125 patients in the treatment group and 115 patients in the placebo group.	Evaluated whether the application of IVIG increases the chances of a successful pregnancy in women with RM. Data from patients enrolled in six different trials were analysed.	There were no significant differences between groups in successful PrR ($P = 0.78$), or LBR ($P = 1.00$).	Daya *et al.* (1999)
	Multicentre, randomised, double-blind, placebo-controlled trial/meta-analysis	82 patients with idiopathic secondary RM.	The effect of IVIG therapy for the treatment of idiopathic secondary RM was evaluated. Patients received IVIG or a saline solution.	LBR did not differ significantly between groups. Furthermore, the meta-analysis performed by including two other randomised-controlled trials also showed no significant effect of treatment with IVIG.	Stephenson *et al.* (2010)
	Randomised comparative study versus placebo	39 patients with: a) two or more very early miscarriages or biochemical pregnancies and b) three or more failed attempts of embryo transfer after IVF.	The efficacy of IVIG treatment to treat implantation failure was assessed based on reproductive outcomes.	PrR was not significantly changed but the IR in the IVIG group was significantly higher compared to the placebo group ($P < 0.05$).	Placido *et al.* (1994)
	Prospective, randomised, double-blind, placebo-controlled trial	95 enrolled women with two or more previous miscarriages.	Efficacy of IVIG therapy to treat RM was evaluated. Patients were classified into two groups, one received IVIG treatment and the other a placebo.	The LBR between the groups was significantly higher in the group that received IVIG therapy ($P = 0.04$).	Coulam *et al.* (1995)
	Prospective, randomised, double-blind, placebo-controlled trial	62 women with a history of two or more documented consecutive spontaneous pregnancy losses.	The efficacy of IVIG therapy for the treatment of RM was evaluated.	There were no statistically significant differences in pregnancy success between groups.	Stephenson *et al.* (1998)

Continued

Table I Continued

Immune therapy	Type of study	Patients	General description (treatment/duration/objectives)	Results/Conclusions	Publication
Intravenous immunoglobulin (IVIG)	Multicentre, double-blind, placebo-controlled study	46 women who experienced RM (three or more miscarriages) were included. 22 IVIG and 24 placebo.	The effect of IVIG therapy on reproductive outcomes was evaluated in women who had three or more miscarriages.	There were no statistically significant differences in the number of pregnancies of more than 12 weeks or in the number of live births between groups.	Perino *et al.* (1997)
	Double-blind, randomised, placebo-controlled study	41 women with RM.	The efficacy of IVIG therapy to prevent RM was evaluated.	There were no statistically significant differences between the IVIG treated group and the placebo group in terms of LBR.	Jablonowska *et al.* (1999)
	Randomised, double-blind, placebo-controlled trial	58 women with at least four unexplained miscarriages.	The effect of infusions of IVIG on women with at least four unexplained miscarriages on pregnancy outcomes was evaluated.	The LBR in women with secondary RM did not show statistically significant differences. However, when including data from a previous placebo-controlled trial, the differences were significant ($P < 0.02$).	Christiansen *et al.* (2002)
	Prospective and randomised clinical trial	76 patients were included in the intralipid group and 78 patients in the IVIG group.	Evaluated whether intralipid therapy can be comparable or an alternative to IVIG treatment.	No statistically significant differences in PrR between the two groups.	Meng *et al.* (2016)
	Retrospective analysis of clinical data	75 women with infertility and elevated cytokines levels in peripheral blood.	Whether treatment with IVIG and TNF-α inhibitors improves IVF success rates was evaluated.	Significant differences were observed in IR, PrR, clinical PrR and LBR between groups ($P < 0.05$).	Winger *et al.* (2009)
	Retrospective study	202 IVF cycles in subfertile women (197 patients).	The efficacy of IVIG therapy was evaluated in immune elevated groups. Women were divided into 4 groups based on the Th1: Th2 ratio and the % of $CD56^+$ cells.	The IR, clinical PrR and LBR were significantly better between groups I and II and between groups II and IV.	Winger *et al.* (2011)
	Observational study	157 patients with RM or RIF after IVF.	The effectiveness of IVIG therapy to treat recurrent reproductive failure was evaluated. The success of the pregnancy after IVIG administration was analysed.	The PrR in women who did not receive IVIG treatment was significantly lower compared to that of women who received IVIG ($P < 0.0001$).	Moraru *et al.* (2012)
	Observational study	8 women with RM.	The effect of IVIG on the activity of pbNK cells was investigated. Women with RM were selected and all of them received 400 mg/kg/day of IVIG for 3 consecutive days.	The results showed an inhibition of NK cell activity and suggest that IVIG treatment could be beneficial for women with RM.	Ruiz *et al.* (1996)
	Systematic review	8 trials involving 442 women.	A systematic review of randomised-controlled trials was conducted to evaluate the effect of IVIG treatment for RM.	After IVIG application, LBR significantly increased in women with secondary RM ($P = 0.04$), but no benefit was seen for women with primary miscarriage.	Hutton *et al.* (2007)
	Systematic review	A total of six randomised-controlled trials evaluating the efficacy of IVIG therapy to treat RM.	A computerized search was carried out in different databases and a randomised-controlled trial.	No benefits were observed. The meta-analysis of the six trials did not suggest an increase in the probability of a live birth with IVIG treatment.	Ata *et al.* (2011)
Intralipid	Double-blind, randomised-controlled study	296 women.	The efficacy of intralipid supplementation in women with RM and elevated natural killer cell activity (>12%) was evaluated. The patients were divided into two groups (one received the intralipid infusion and the other saline infusion).	Statistically significant differences were observed in the frequencies of ongoing pregnancies and live births ($P = 0.005$ for both) between the two groups.	Dakhly *et al.* (2016)
	Randomised-controlled trial	142 patients with RIF.	Reproductive outcomes were evaluated after intralipid infusion administration in 142 patients with RIF. They were divided into two groups, with and without treatment.	LBR between groups did not show statistically significant differences.	Al-Zebeidi *et al.* (2020)

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Table I Continued

Immune therapy	Type of study	Patients	General description (treatment/duration/objectives)	Results/Conclusions	Publication
Intralipid	Retrospective cohort study	127 patients with RM or RIF.	Evaluated if intralipid infusions improve the reproductive results of women with RM or RIF. LBRs were compared between a group that received the treatment and a control group.	Both the number of clinical pregnancies and the LBR did not show statistically significant differences ($P = 0.12$ and 0.80, respectively) when the results were compared between the groups.	Martini *et al.* (2018)
	Retrospective cohort study	187 women with RIF or RM.	The efficacy of intralipid therapy to treat RM and RIF was evaluated.	LBR was significantly higher ($P = 0.049$) in women who received intralipid therapy.	Plaçais *et al.* (2020)
G-CSF	Randomised-controlled trial	68 patients with unexplained RM (with at least four consecutive spontaneous abortions).	The effectiveness of G-CSF treatment to prevent RM was evaluated.	LBR in women treated with G-CSF was significantly higher compared to the placebo group ($P = 0.0061$). The levels of β-hCG were significantly higher in women treated with G-CSF versus placebo ($P < 0.001$).	Scarpellini and Sbracia (2009)
	Randomised-controlled clinical trial	100 infertile women.	The effects of G-CSF administration on reproductive outcomes after normal IVF treatment were evaluated. IVF results were compared between the treated group and the control group.	G-CSF did not improve pregnancy outcomes. No significant differences between groups in PrR, IR and miscarriage rate were found.	Eftekhar *et al.* (2016)
	Randomised clinical trial	68 patients with two or three miscarriages.	The participants in this randomised trial were divided into two groups: a control group and a group that received an intrauterine injection of G-CSF.	No significant differences were found in IR or PrR.	Zafardoust *et al.* (2017)
	Randomised, double-blind, placebo-controlled clinical trial	150 women with a history of unexplained RM.	Evaluated whether the administration of recombinant human G-CSF improves reproductive results in women with unexplained RM.	No significant differences were observed between treated and placebo groups for any outcomes.	Eapen *et al.* (2019)
	Retrospective cohort study	127 patients with RM and 78 controls.	It was verified whether the administration of G-CSF improved reproductive results in patients with RM compared to two subgroups without G-CSF treatment (one with another medication and the other without medication).	A significantly higher PrR and LBR ($P = 0.016$; $P = 0.006$; $P = 0.016$; $P = 0.016$) was observed when comparing the G-CSF group with the two subgroups without G-CSF treatment. Some adverse effects were recorded in less than 10% of patients	Santjohanser *et al.* (2013)
Various treatments	Systematic review and meta-analysis	30 publications were included.	A comprehensive systematic review and a meta-analysis were performed to study the current evidence for IVF immunotherapy as a treatment for RM and RIF.	No improvement in LBR or RM prevention was observed after the application of immune therapies.	Achilli *et al.* (2018)
	Review	Twenty trials were included. Randomised trials of immunotherapies used to treat women with RM (with three or more miscarriages) and no more than one live birth were included.	This review evaluates the effects of different immune treatments (leukocyte immunization, IVIG, intralipid, etc.) on the LBR in women with previous unexplained RM.	Paternal cell immunization, third party donor leukocytes, trophoblast membranes, and IVIG provide no significant beneficial effect over placebo in preventing further miscarriages.	Scott (2003)

G-CSF, granulocyte colony-stimulating factor; IVIG, intravenous immunoglobulin; IR, implantation rate; LBR, live birth rate; NK, natural killer cells; LMWH, low molecular weight heparin; PrR, pregnancy rate; RIF, recurrent implantation failure; RM, recurrent miscarriage.

treatments (Lee *et al.*, 1994; Moffitt *et al.*, 1995; Ubaldi *et al.*, 2002; Duvan *et al.*, 2006). In a case-control study, the effectiveness of combined co-treatment with aspirin, doxycycline and prednisolone in IVF and ICSI cycles was investigated by evaluating the live birth rate. However, no significant differences were found in the cycles with fresh embryo transfers, (Motteram *et al.*, 2015). In frozen embryo transfer cycles, the live birth rate was even lower in the group given the combined adjuvant (Motteram *et al.*, 2015). Criticisms have been raised about these studies because of the differences in corticosteroid administration protocols, types and doses of drugs used (prednisolone, methylprednisolone, dexamethasone and hydrocortisone combined with prednisolone), and the treatment duration (Boomsma *et al.*, 2012).

Another aspect to consider is the lack of consensus when considering the levels of reference uNK cells (or pbNK cells). There is no established cut-off value to define an 'abnormal' level of uNK cells. Some studies consider a 'high' level of uNK cells as >5% of total stromal cells (Quenby *et al.*, 2005), while others have established a cut-off value of 13.8% using the 90th percentile as the upper limit of normality (Tuckerman *et al.*, 2007). There is no consolidated value when evaluating uNK cells as a diagnostic tool. For a cohort of 215 women, mean percentages of uNK cells significantly differed at 2.5% in fertile women, 3.2% in women with RM, and 3.1% in women with RIF (Chen *et al.*, 2017b). In addition, when establishing a lower limit of 1.2%, 16% of women with RM and 18% with RIF had uNK cells below this value (Chen *et al.*, 2017b). Most studies focused on evaluating higher levels of uNK cells without paying attention to lower levels, thus few studies have evaluated possible detrimental effects on the endometrium of such low levels.

Although some treatments have achieved favourable reproductive results, many other studies showed no benefit. Therefore, more well-designed prospective randomised studies with larger sample sizes are necessary to assess the efficacy, reliability and safety of this type of immunotherapy in a more objective and complete way. Furthermore, some treatments, such as IVIG therapy, apart from being financially costly also have reported cases of adverse reactions, some of them severe (Duhem *et al.*, 1994). A meta-analysis including nine randomised controlled trials to check whether alloimmune stimulation of the female partner improves live birth rate concluded that paternal cell immunisation may be an effective treatment. However, although the treatment effect appears to be small (helping only 8% to 10% of couples), significantly more problems were observed among immunised women, including hepatitis, cytomegalovirus, flu-like symptoms and transfusion reactions. Some patients also developed antipaternal erythrocyte antibodies, antipaternal platelet antibodies or autoimmune problems (Coulam *et al.*, 1994). The FDA banned LIT in 2002 considering this treatment as a new investigational drug with significant associated safety concerns.

However, not all patients with RM have high levels of uNK cells, so application of this type of therapy may not have the desired effect. While personalized treatments adapted to each individual case are an alternative, such strategies are complicated by variability in the number of uNK cells throughout the menstrual cycle (Wilkens *et al.*, 2013) as well as the lack of a standardized protocol or test across clinics (Moffett and Shreeve, 2015).

A recent meta-analysis evaluated the effectiveness of different immune therapies for the treatment of RM. The most common immunomodulators, such as immunoglobulin, LIT, intralipid and intrauterine infusion of G-CSF, were evaluated (Achilli *et al.*, 2018). This complete and exhaustive review of the available medical literature on the role of immunotherapy in reproductive outcomes concluded that immunotherapies should only be applied in the context of research. This is because of the lack of evidence showing any clear improvement in RM prevention or in the live birth rates in women undergoing IVF. There is a need for well-designed randomised controlled trials to correctly address this issue and offer conclusive results (Achilli *et al.*, 2018).

Many studies have established in patients a baseline value of pbNK >12% in order to consider treating them with an immunotherapy (Woon *et al.*, 2020). This, together with the variety of immune therapies being applied without a common criterion, generates even more heterogeneity (Woon *et al.*, 2020). Treatments based on uNK cells must be considered carefully: there is still an intense debate about these therapies, many doctors affirm that these types of therapies offer false expectations to patients, the options are mostly expensive and, on occasion, they have been associated with side effects. For this reason, these treatments should not continue to be offered to treat unexplained recurrent pregnancy loss. Furthermore, the studies showing beneficial results do not provide predictive value for the success of pregnancy and should be abandoned (Scott, 2003).

Conclusion

One of the issues that this review presents is the enormous complexity involved in studying the immune system in reproduction. It is practically impossible to evaluate each element in isolation, because of interactions with the other components. The complexity in the immunology of the maternal–foetal interface lies in the great variety of participating molecules, the processes and interactions that occur at different levels (molecular, cellular, tissue, etc.) and the great diversity of genetic combinations that are translated into different types of responses.

This review highlights the critical and important role that uNK cells play during pregnancy. Indeed, these cells perform their main functions during the most critical phases of pregnancy, that is, in its early stages. Although uNK cells have been regarded as 'enemies' in reproduction, it seems that they are more allies than was previously thought. Until recently, uNK cells were thought to be equivalent to cytotoxic pbNK cells, in part owing to their name. But, as discussed throughout the article, uNK cells are not implicated in a cytotoxic response against the embryo. Recently obtained knowledge of these cells in the endometrium during the menstrual cycle, especially in the decidua, shows that they are instead key regulators in the early stages of pregnancy, playing a fundamental role in vascular remodeling and trophoblast invasion. Thus, alterations in their functions could compromise reproductive success. Furthermore, the study of uNK KIR receptors and their interaction with trophoblast HLA-C could help clinicians to prevent pregnancy disorders such as miscarriage, pre-eclampsia and low birthweight. This could be quite a breakthrough for IVF clinics, as oocyte or sperm donors for patients with the KIR AA genotype could be selected based on their HLA-C expression. Similarly, the embryos to be transferred to patients with this haplotype who undergo RM or RIF could be selected, also based on their HLA-C expression (Alecsandru *et al.*, 2014, 2020).

Additional research is needed to establish the functions, interactions and processes in which uNK cells participate, since their clinical implications could be of great interest and beneficial for many women worldwide. Couples in which the woman has a KIR AA genotype and her male partner is homozygous for HLA-C2 have a more negative prognosis. Thus, the use of sperm/oocytes from a homozygous HLA-C1 donor could be considered along with single embryo transfer (Hiby *et al.*, 2008; Alecsandru *et al.*, 2014, 2020). Translation of this idea into clinical practice could lead to improved live birth rate for affected KIR AA patients (Alecsandru *et al.*, 2020).

One of the main problems in studying reproductive immunology is that of considering results obtained from the concentration and activity of pbNK cells as valid for use as a diagnostic test or a predictor of pregnancy outcome (Matsubayashi *et al.*, 2005). Indeed, despite the lack of verified and complete information about the functions of uNK cells and their role in the correct development of pregnancy, many immune treatments and therapies have been offered to patients to avoid RM, RIF or problems such as pre-eclampsia 'caused' by a high number or activity of NK cells. Many of these therapies have been applied without empirical evidence to back them up, and often consist of expensive tests that do not produce the desired results. These studies show controversial results because the number of included patients is not always large enough for sufficiently strong statistical power to be able to draw clear and consistent conclusions. Likewise, there is great variability in terms of inclusion criteria, the methodology followed to analyse NK cells, outcome measures, and the doses, duration, or types of medications used between different studies. This heterogeneity decreases the value of the studies and requires the results to be interpreted with extreme caution. Therefore, due to the lack of information and evidence supporting the use of immune therapies in IVF patients, the discrepancies between the studies themselves and the lack of consensus, efficacy and safety, whether these treatments should be offered to couples undergoing ART has been highly questioned. Specific tests are needed to safely study and diagnose RM caused by immune system disorders. Some of the unexplained early losses are likely due to genetic abnormalities that have not yet been defined and that may be affecting early embryo development (Copp, 1995; Pegoraro *et al.*, 1997; Spandidos *et al.*, 1998; Tsai *et al.*, 1998; Rossant and Cross, 2001; Tempfer *et al.*, 2001; Quenby *et al.*, 2002). In turn, to evaluate the efficacy of any proposed treatment, it is essential to carry out randomised-controlled studies with an adequate number of participants. Complete, well-designed studies with strong statistical power and common methodology (to reduce variability between studies) are necessary to provide conclusive scientific evidence. This would allow evaluation of the real benefit, if any, of these therapies in terms of live births after IVF application, ensuring the health and safety of the patient.

Data availability

No new data were generated or analysed in support of this research.

Authors' roles

I.D. made the compilation, reading and analysis of the bibliography. She was in charge of writing the review. She also designed the figures and the table. F.D. coordinated, contributed and revised the writing and most of the referenced research. He has also been essential in all revisions of the document. J.A.G.V. and D.A. also contributed to the correction and verification of the information and have been essential in the final revision of the document.

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Conflict of interest

The authors declare no conflict of interest.

References

Acar N, Ustunel I, Demir R. Uterine natural killer (uNK) cells and their missions during pregnancy: a review. *Acta Histochem* 2011; **113**:82–91.

Achilli C, Duran-Retamal M, Saab W, Serhal P, Seshadri S. The role of immunotherapy in in vitro fertilization and recurrent pregnancy loss: a systematic review and meta-analysis. *Fertil Steril* 2018;**110**: 1089–1100.

Alan E. Beer Centre. Centre for reproductive immunology and genetics. 2015. http://www.repro-med.net/ (10 April 2020, date last accessed).

Alecsandru D, Barrio A, Garrido N, Aparicio P, Pellicer A, Moffett A, García-Velasco JA. Parental human leukocyte antigen-C allotypes are predictive of live birth rate and risk of poor placentation in assisted reproductive treatment. *Fertil Steril* 2020;**114**:809–817.

Alecsandru D, Garciá-Velasco JA. Immunology and human reproduction. *Curr Opin Obstet Gynecol* 2015;**27**:231–234.

Alecsandru D, Garcia-Velasco JA. Is there a role for human leukocyte antigen-G typing in infertility treatment? *Fertil Sterility* 2020; **114**:515–516.

Alecsandru D, García-Velasco JA. Why natural killer cells are not enough: a further understanding of killer immunoglobulin-like receptor and human leukocyte antigen. *Fertil Steril* 2017;**107**: 1273–1278.

Alecsandru D, Garrido N, Vicario JL, Barrio A, Aparicio P, Requena A, García-Velasco JA. Maternal KIR haplotype influences live birth rate after double embryo transfer in IVF cycles in patients with recurrent miscarriages and implantation failure. *Hum Reprod* 2014; **29**:2637–2643.

Alhalabi M, Samawi S, Taha A, Kafri N, Modi S, Khatib A, Sharif J, Othman A. Prednisolone improves implantation in ICSI patients with high peripheral CD69 + NK Cells. *Hum Reprod* 2011;**26** Suppl 1:i291.Abstract no: P-249.

Allan DSJ, Colonna M, Lanier LL, Churakova TD, Abrams JS, Ellis SA, McMichael AJ, Braud VM. Tetrameric complexes of human

histocompatibility leukocyte antigen (HLA)-G bind to peripheral blood myelomonocytic cells. *J Exp Med* 1999;**189**:1149–1155.

Al-Zebeidi J, Agdi M, Lary S, Al-Obaid S, Salim G, Al-Jaroudi D. Effect of empiric intravenous intralipid therapy on pregnancy outcome in women with unexplained recurrent implantation failure undergoing intracytoplasmic sperm injection-embryo transfer cycle: a randomized controlled trial. *Gynecol Endocrinol* 2020;**36**: 131–134.

Antony Nolan Research Institute. HLA Nomenclature. 2020. http://hla.alleles.org/nomenclature/stats.html. (11 September 2020, date last accessed).

Apps R, Gardner L, Hiby SE, Sharkey AM, Moffett A. Conformation of human leucocyte antigen-C molecules at the surface of human trophoblast cells. *Immunology* 2008;**124**:322–328.

Apps R, Murphy SP, Fernando R, Gardner L, Ahad T, Moffett A. Human leucocyte antigen (HLA) expression of primary trophoblast cells and placental cell lines, determined using single antigen beads to characterize allotype specificities of anti-HLA antibodies. *Immunology* 2009;**127**:26–39.

Arck PC, Hecher K. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. *Nat Med* 2013;**19**: 548–556.

Arruvito L, Giulianelli S, Flores AC, Paladino N, Barboza M, Lanari C, Fainboim L. NK cells expressing a progesterone receptor are susceptible to progesterone-induced apoptosis. *J Immunol* 2008; **180**:5746–5753.

Ata B, Tan SL, Shehata F, Holzer H, Buckett W. A systematic review of intravenous immunoglobulin for treatment of unexplained recurrent miscarriage. *Fertil Steril* 2011;**95**:1080–1085.e2.

Ball E, Bulmer JN, Ayis S, Lyall F, Robson SC. Late sporadic miscarriage is associated with abnormalities in spiral artery transformation and trophoblast invasion. *J Pathol* 2006;**208**:535–542.

Becknell B, Caligiuri MA. Interleukin-2, interleukin-15, and their roles in human natural killer cells. *Adv Immunol* 2005;**86**:209–239.

Biassoni R, Cantoni C, Falco M, Verdiani S, Bottino C, Vitale M, Conte R, Poggi A, Moretta A, Moretta L. The human leukocyte antigen (HLA)-C-specific "activatory" or "inhibitory" natural killer cell receptors display highly homologous extracellular domains but differ in their transmembrane and intracytoplasmic portions. *J Exp Med* 1996;**183**:645–650.

Bischof P, Irminger-Finger I. The human cytotrophoblastic cell, a mononuclear chameleon. *Int J Biochem Cell Biol* 2005;**37**:1–16.

Biswas Shivhare S, Bulmer JN, Innes BA, Hapangama DK, Lash GE. Menstrual cycle distribution of uterine natural killer cells is altered in heavy menstrual bleeding. *J Reprod Immunol* 2015;**112**:88–94.

Boomsma CM, Keay SD, Macklon NS. Peri-implantation glucocorticoid administration for assisted reproductive technology cycles. *Cochrane Database Syst Rev* 2012;CD005996.

Braud V, Jones EY, McMichael A. The human major histocompatibility complex class Ib molecule HLA-E binds signal sequence-derived peptides with primary anchor residues at positions 2 and 9. *Eur J Immunol* 1997;**27**:1164–1169.

Braud VM, Allan DSJ, O'Callaghan CA, Söderström K, D'Andrea A, Ogg GS, Lazetic S, Young NT, Bell JI, Phillips JH *et al.* HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 1998a;**391**:795–799.

Braud VM, Allan DSJ, Wilson D, McMichael AJ. TAP- and tapasin-dependent HLA-E surface expression correlates with the binding of an MHC class I leader peptide. *Curr Biol* 1998b;**8**:1–10.

Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;**204**:193–201.

Brosens IA. Morphological changes in the utero-placental bed in pregnancy hypertension. *Clin Obstet Gynecol* 1977;**4**:573–593.

Bulmer JN, Hollings D, Ritson A. Immunocytochemical evidence that endometrial stromal granulocytes are granulated lymphocytes. *J Pathol* 1987;**153**:281–288.

Bulmer JN, Innes BA, Robson SC, Lash GE. Transient loss of endothelial cells in human spiral artery remodelling during early pregnancy: Challenging the dogma. *Placenta* 2020;**101**:230–233.

Bulmer JN, Lash GE. Human uterine natural killer cells: a reappraisal. *Mol Immunol* 2005;**42**:511–521.

Bulmer JN, Williams PJ, Lash GE. Immune cells in the placental bed. *Int J Dev Biol* 2010;**54**:281–294.

Burton GJ, Woods AW, Jauniaux E, Kingdom JCP. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. *Placenta* 2009;**30**:473–482.

Caligiuri MA. Human natural killer cells. *Blood* 2008;**112**:461–469.

Campbell JJ, Qin S, Unutmaz D, Soler D, Murphy KE, Hodge MR, Wu L, Butcher EC. Unique subpopulations of CD56 + NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. *J Immunol* 2001;**166**:6477–6482.

Carp HJA, Toder V, Torchinsky A, Portuguese S, Lipitz S, Gazit E, Mashiach S, Coulam CB, Clark DA, Aoki K *et al.* Allogenic leukocyte immunization after five or more miscarriages. *Hum Reprod* 1997;**12**:250–255.

Carter AM, Pijnenborg R. Evolution of invasive placentation with special reference to non-human primates. *Best Pract Res Clin Obstet Gynaecol* 2011;**25**:249–257.

Cauchi MN, Lim D, Young DE, Kloss M, Pepperell RJ. Treatment of recurrent aborters by immunization with paternal cells—controlled trial. *Am J Reprod Immunol* 1991;**25**:16–17.

Chakraborty D, Rumi MAK, Konno T, Soares MJ. Natural killer cells direct hemochorial placentation by regulating hypoxia-inducible factor dependent trophoblast lineage decisions. *Proc Natl Acad Sci U S A* 2011;**108**:16295–16300.

Chantakru S, Miller C, Roach LE, Kuziel WA, Maeda N, Wang W-C, Evans SS, Croy BA. Contributions from self-renewal and trafficking to the uterine NK cell population of early pregnancy. *J Immunol* 2002;**168**:22–28.

Chao K-H, Yang Y-S, Ho G-N, Chen S-U, Chen H-F, Dai H-J, Huang S-C, Gill TJ. Decidual natural killer cytotoxicity decreased in normal pregnancy but not in anembryonic pregnancy and recurrent spontaneous abortion. *Am J Reprod Immunol* 1995;**34**:274–280.

Chazara O, Xiong S, Moffett A. Maternal KIR and fetal HLA-C: a fine balance. *J Leukoc Biol* 2011;**90**:703–716.

Check JH, Check DL. Intravenous intralipid therapy is not beneficial in having a live delivery in women aged 40-42 years with a previous history of miscarriage or failure To conceive despite embryo transfer undergoing in vitro fertilization-embryo transfer. *Clin Exp Obstet Gynecol* 2016;**43**:14–15.

Chen C, Danekas LH, Ratko TA, Vlasses PH, Matuszewski KA. A multicenter drug use surveillance of intravenous immunoglobulin utilization in US academic health centers. *Ann Pharmacother* 2000; **34**:295–299.

Chen X, Jiang L, Wang CC, Huang J, Li TC. Hypoxia inducible factor and microvessels in peri-implantation endometrium of women with recurrent miscarriage. *Fertil Steril* 2016a;**105**:1496–1502.e4.

Chen X, Liu Y, Jiang L, Wang C, Li T. Angiogenic cytokine profiles of human uterine CD56+ natural killer cells in women with recurrent reproductive failure. *Am J Reprod Immunol* 2016b;**67**:278–286.

Chen X, Man GCW, Liu Y, Wu F, Huang J, Li TC, Wang CC. Physiological and pathological angiogenesis in endometrium at the time of embryo implantation. *Am J Reprod Immunol* 2017a;**78**: e12693.

Chen X, Mariee N, Jiang L, Liu Y, Wang CC, Li TC, Laird S. Measurement of uterine natural killer cell percentage in the periimplantation endometrium from fertile women and women with recurrent reproductive failure: establishment of a reference range. *Am J Obstet Gynecol* 2017b;**217**:680.e1–680.e6.

Chiossone L, Vacca P, Orecchia P, Croxatto D, Damonte P, Astigiano S, Barbieri O, Bottino C, Moretta L, Mingari MC. In vivo generation of decidual natural killer cells from resident hematopoietic progenitors. *Haematologica* 2014;**99**:448–457.

Christiansen OB, Mathiesen O, Husth M, Lauritsen JG, Grunnet N. Placebo-controlled trial of active immunization with third party leukocytes in recurrent miscarriage. *Acta Obstet Gynecol Scand* 1994; **73**:261–268.

Christiansen OB, Pedersen B, Rosgaard A, Husth M. A randomized, double-blind, placebo-controlled trial of intravenous immunoglobulin in the prevention of recurrent miscarriage: evidence for a therapeutic effect in women with secondary recurrent miscarriage. *Hum Reprod* 2002;**17**:809–816.

Clifford K, Flanagan AM, Regan L. Endometrial CD56 + natural killer cells in women with recurrent miscarriage: a histomorphometric study. *Hum Reprod* 1999;**14**:2727–2730.

Colonna M, Navarro F, Bellón T, Llano M, García P, Samaridis J, Angman L, Cella M, López-Botet M. A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *J Exp Med* 1997;**186**: 1809–1818.

Colonna M, Samaridis J. Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells. *Science* 1995;**268**:405–408.

Colucci F. The role of KIR and HLA interactions in pregnancy complications. *Immunogenetics* 2017;**69**:557–565.

Constantine MM, Thomas W, Whitman L, Kahwash E, Dolan S, Smith S, Caudle CJ, Burton E, Anderson DR. Intravenous immunoglobulin utilization in the Canadian Atlantic provinces: a report of the Atlantic Collaborative Intravenous Immune Globulin Utilization Working Group. *Transfusion* 2007;**47**:2072–2080.

Cooley S, Verneris MR, Miller JS. Natural killer cell-based therapies. In: *Hematology: Basic Principles and Practice*. Elsevier Inc, 2018, 1575–1582.

Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001;**22**:633–640.

Copp AJ. Death before birth: clues from gene knockouts and mutations. *Trends Genet* 1995;**11**:87–93.

Coulam CB, Clark DA, Collins J, Scott JR, Schlesselman JS, Aoki K, Carp HJA, Cauchi MN, Lim D, Christiansen OB. Worldwide collaborative observational study and meta-analysis on allogenic leukocyte immunotherapy for recurrent spontaneous abortion. *Am J Reprod Immunol* 1994;**32**:55–72.

Coulam CB, Krysa L, Stern JJ, Bustillo M. Intravenous immunoglobulin for treatment of recurrent pregnancy loss. *Am J Reprod Immunol* 1995;**34**:333–337.

Croy BA, Esadeg S, Chantakru S, Van Den Heuvel M, Paffaro VA, He H, Black GP, Ashkar AA, Kiso Y, Zhang J. Update on pathways regulating the activation of uterine Natural Killer cells, their interactions with decidual spiral arteries and homing of their precursors to the uterus. *J Reprod Immunol* 2003;**59**:175–191.

Dakhly DMR, Bayoumi YA, Sharkawy M, Allah G, Hassan SH, Gouda MA, Hashem HM, Hatem AT, Ahmed DL, El-Khayat MF. W. Intradlipid supplementation in women with recurrent spontaneous abortion and elevated levels of natural killer cells. *Int J Gynecol Obstet* 2016;**135**:324–327.

Dan S, Wei W, Yichao S, Hongbo C, Shenmin Y, Jiaxiong W, Hong L. Effect of prednisolone administration on patients with unexplained recurrent miscarriage and in routine intracytoplasmic sperm injection: a meta-analysis. *Am J Reprod Immunol* 2015;**74**: 89–97.

Darmochwal-Kolarz D, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, Oleszczuk J. The concentrations of soluble HLA-G protein are elevated during mid-gestation and decreased in pre-eclampsia. *Folia Histochem Cytobiol* 2012;**50**:286–291.

Das M, Holzer HEG. Recurrent implantation failure: gamete and embryo factors. *Fertil Steril* 2012;**97**:1021–1027.

Daya S, Gunby J, Clark DA. Intravenous immunoglobulin therapy for recurrent spontaneous abortion: a meta-analysis. *Am J Reprod Immunol* 1998;**39**:69–76.

Daya S, Gunby J. The effectiveness of allogeneic leukocyte immunization in unexplained primary recurrent spontaneous abortion. *Am J Reprod Immunol* 1994;**32**:294–302.

Daya S, Gunby J, Porter F, Scott J, Clark DA. Critical analysis of intravenous immunoglobulin therapy for recurrent miscarriage. *Hum Reprod Update* 1999;**5**:475–482.

De Agustín P, López-Rubio F, Picazo ML, Segura A. Vascular disorders in habitual abortion. *Acta Obstet Ginecol Hisp Lusit* 1971;**19**: 475–488.

De Fried EP, Blanco L, Lancuba S, Asch RH. Improvement of clinical pregnancy rate and implantation rate of in-vitro fertilization-embryo transfer patients by using methylprednisone. *Hum Reprod* 1993;**8**:393–395.

Driver I, Feather JW, King PR, Dawson JB. The optical properties of aqueous suspensions of Intralipid, a fat emulsion. *Phys Med Biol* 1989;**34**:1927–1930.

Duhem C, Dicato MA, Ries F. Side-effects of intravenous immune globulins. *Clinical and Experimental Immunology, Supplement 1*, **1994**;**97**:79–83.

Duvan CI, Ozmen B, Satıroglu H, Atabekoglu CS, Berker B. Does addition of low-dose aspirin and/or steroid as a standard treatment in nonselected intracytoplasmic sperm injection cycles improve in vitro fertilization success? A randomized, prospective, placebo-controlled study. *J Assist Reprod Genet* 2006;**23**:15–21.

Eapen A, Joing M, Kwon P, Tong J, Maneta E, De Santo C, Mussai F, Lissauer D, Carter D, Ahmed A *et al*. Recombinant human granulocyte-colony stimulating factor in women with unexplained recurrent pregnancy losses: a randomized clinical trial. *Hum Reprod* 2019;**34**:424–432.

Eftekhar M, Hosseinisadat R, Baradaran R, Naghshineh E. Effect of granulocyte colony stimulating factor (G-CSF) on IVF outcomes in infertile women: an RCT. *Int J Reprod Biomed* 2016;**14**:341–346.

Ehrlich R, Hull ML, Walkley J, Sacks G. Intralipid immunotherapy for repeated IVF failure. *FandR* 2019;**01**:154–160.

Ellis SA, Palmer MS, McMichael AJ. Human trophoblast and the choriocarcinoma cell line BeWo express a truncated HLA class I molecule. *J Immunol* 1990;**144**:731–735.

Eriksson M, Meadows SK, Wira CR, Sentman CL. Unique phenotype of human uterine NK cells and their regulation by endogenous TGF-β. *J Leukoc Biol* 2004;**76**:667–675.

Faridi RM, Agrawal S. Killer immunoglobulin-like receptors (KIRs) and HLA-C allorecognition patterns implicative of dominant activation of natural killer cells contribute to recurrent miscarriages. *Hum Reprod* 2011;**26**:491–497.

Fawzy M, Shokeir T, El-Tatongy M, Warda O, El-Refaiey AAA, Mosbah A. Treatment options and pregnancy outcome in women with idiopathic recurrent miscarriage: a randomized placebo-controlled study. *Arch Gynecol Obstet* 2008;**278**:33–38.

Fernandez N, Cooper J, Sprinks M, Abdelrahman M, Fiszer D, Kurpisz M, Dealtry G. A critical review of the role of the major histocompatibility complex in fertilization, preimplantation development and feto-maternal interactions. *Hum Reprod Update* 1999;**5**:234–248.

Flaherty DK. Natural killer cells. In: *Immunology for Pharmacy*. New York, NY: Mosby, 2012, 169–174.

Fraser EJ, Grimes DA, Schulz KF. Immunization as therapy for recurrent spontaneous abortion: a review and meta-analysis. *Obstet Gynecol* 1994;**83**:637–638.

Fraser R, Whitley GS, Johnstone AP, Host AJ, Sebire NJ, Thilaganathan B, Cartwright JE. Impaired decidual natural killer cell regulation of vascular remodelling in early human pregnancies with high uterine artery resistance. *J Pathol* 2012;**228**:322–332.

Gatenby PA, Cameron K, Simes RJ, Adelstein S, Bennett MJ, Jansen RPS, Shearman RP, Stewart GJ, Whittle M, Doran TJ. Treatment of recurrent spontaneous abortion by immunization with paternal lymphocytes: Results of a controlled trial. *Am J Reprod Immunol* 1993;**29**:88–94.

Genbacev O, Schubach SA, Miller RK. Villous culture of first trimester human placenta-model to study extravillous trophoblast (EVT) differentiation. *Placenta* 1992;**13**:439–461.

Geraghty DE, Koller BH, Orr HT. A human major histocompatibility complex class I gene that encodes a protein with a shortened cytoplasmic segment. *Proc Natl Acad Sci U S A* 1987;**84**:9145–9149.

Geraghty DE, Wei X, Orr HT, Koller BH. Human leukocyte antigen F (HLA-F): an expressed HLA gene composed of a class I coding sequence linked to a novel transcribed repetitive element. *J Exp Med* 1990;**171**:1–18.

Gomaa MF, Elkholy AG, El-Said MM, Abdel-Salam NE. Combined oral prednisolone and heparin versus heparin: the effect on peripheral NK cells and clinical outcome in patients with unexplained recurrent miscarriage. A double-blind placebo randomized controlled trial. *Arch Gynecol Obstet* 2014;**290**:757–762.

Granato D, Blum S, Rossle C, Le Boucher J, Malnoe A, Dutot G. Effects of parenteral lipid emulsions with different fatty acid composition on immune cell functions in vitro. *JPEN J Parenter Enteral Nutr* 2000;**24**:113–118.

Gubbay O, Critchley HOD, Bowen JM, King A, Jabbour HN. Prolactin induces ERK phosphorylation in epithelial and CD56+ natural killer cells of the human endometrium. *J Clin Endocrinol Metab* 2002;**87**:2329–2335.

Guillaudeux T, Rodriguez AM, Girr M, Mallet V, Ellis SA, Sargent IL, Fauchet R, Alsat E, Le Bouteiller P. Methylation status and transcriptional expression of the MHC class I loci in human trophoblast cells from term placenta. *J Immunol* 1995;**154**:3283–3299.

Hackmon R, Koifman A, Hyobo H, Glickman H, Sheiner E, Geraghty DE. Reduced third-trimester levels of soluble human leukocyte antigen G protein in severe preeclampsia. *Am J Obstet Gynecol* 2007;**197**:255.e1–255.e5.

Hackmon R, Pinnaduwage L, Zhang J, Lye SJ, Geraghty DE, Dunk CE. Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. *Am J Reprod Immunol* 2017;**77**:e12643.

Hameed A, Fox WM, Kurman RJ, Hruban RH, Podack ER. Perforin expression in endometrium during the menstrual cycle. *Int J Gynecol Pathol* 1995;**14**:143–150.

Handgretinger R, Lang P, André MC. Exploitation of natural killer cells for the treatment of acute leukemia. *Blood* 2016;**127**:3341–3349.

Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I *et al*. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 2006;**12**:1065–1074.

Hanna J, Wald O, Goldman-Wohl D, Prus D, Markel G, Gazit R, Katz G, Haimov-Kochman R, Fujii N, Yagel S *et al*. CXCL12 expression by invasive trophoblasts induces the specific migration of CD16- human natural killer cells. *Blood* 2003;**102**:1569–1577.

Hannan NJ, Jones RL, Critchley HOD, Kovacs GJ, Rogers PAW, Affandi B, Salamonsen LA. Coexpression of fractalkine and its receptor in normal human endometrium and in endometrium from users of progestin-only contraception supports a role for fractalkine in leukocyte recruitment and endometrial remodeling. *J Clin Endocrinol Metab* 2004;**89**:6119–6129.

Henderson TA, Saunders PTK, Moffett-King A, Groome NP, Critchley HOD. Steroid receptor expression in uterine natural killer cells. *J Clin Endocrinol Metab* 2003;**88**:440–449.

Hiby SE, Apps R, Chazara O, Farrell LE, Magnus P, Trogstad L, Gjessing HK, Carrington M, Moffett A. Maternal KIR in Combination with Paternal HLA-C2 Regulate Human Birth Weight. *J Immunol* 2014;**192**:5069–5073.

Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, Claas FH, Walker JJ, Redman CC, Morgan L *et al*. Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. *J Clin Invest* 2010a;**120**:4102–4110.

Hiby SE, Ashrafian-Bonab M, Farrell L, Single RM, Balloux F, Carrington M, Moffett A. Distribution of killer cell immunoglobulin-

like receptors (KIR) and their HLA-C ligands in two Iranian populations. *Immunogenetics* 2010b;**62**:65–73.

Hiby SE, King A, Sharkey A, Loke YW. Molecular studies of trophoblast HLA-G: polymorphism, isoforms, imprinting and expression in preimplantation embryo. *Tissue Antigens* 1999;**53**:1–13.

Hiby SE, King A, Sharkey AM, Loke YW. Human uterine NK cells have a similar repertoire of killer inhibitory and activatory receptors to those found in blood, as demonstrated by RT-PCR and sequencing. *Mol Immunol* 1997;**34**:419–430.

Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. *Hum Reprod* 2008;**23**:972–976.

Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CWG, Carrington M, Trowsdale J, Moffett A. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med* 2004;**200**:957–965.

Hickey M, Crewe J, Goodridge JP, Witt CS, Fraser IS, Doherty D, Christiansen FT, Salamonsen LA. Menopausal hormone therapy and irregular endometrial bleeding: a potential role for uterine natural killer cells? *J Clin Endocrinol Metab* 2005;**90**:5528–5535.

Ho H-N-N, Gill TJ, Hsieh H-J-J, Jiang J-J-J, Lee T-Y-Y, Hsieh C-Y-Y. Immunotherapy for recurrent spontaneous abortions in a Chinese population. *Am J Reprod Immunol* 1991;**25**:10–15.

Hofmann AP, Gerber SA, Croy BA. Uterine natural killer cells pace early development of mouse decidua basalis. *Mol Hum Reprod* 2014;**20**:66–76.

Hong Y, Wang X, Lu P, Song Y, Lin Q. Killer immunoglobulin-like receptor repertoire on uterine natural killer cell subsets in women with recurrent spontaneous abortions. *Eur J Obstet Gynecol Reprod Biol* 2008;**140**:218–223.

Hornung D, Ryan IP, Chao VA, Vigne J-L, Schriock ED, Taylor RN. Immunolocalization and regulation of the chemokine RANTES in human endometrial and endometriosis tissues and cells 1. *J Clin Endocrinol Metab* 1997;**82**:1621–1628.

Hutton B, Sharma R, Fergusson D, Tinmouth A, Hebert P, Jamieson J, Walker M. Use of intravenous immunoglobulin for treatment of recurrent miscarriage: a systematic review. *BJOG* 2007;**114**:134–142.

Hviid TVF. HLA-G genotype is associated with fetoplacental growth. *Hum Immunol* 2004;**65**:586–593.

Hviid TVF. HLA-G in human reproduction: aspects of genetics, function and pregnancy complications. *Hum Reprod Update* 2006;**12**:209–232.

Illeni MT, Marelli G, Parazzini F, Acaia B, Bocciolone L, Bontempelli M, Faden D, Fedele L, Maffeis A, Radici E. Immunology: Immunotherapy and recurrent abortion: a randomized clinical trial. *Hum Reprod* 1994;**9**:1247–1249.

Ingman WV, Robertson SA. Defining the actions of transforming growth factor beta in reproduction. *BioEssays* 2002;**24**:904–914.

Inngjerdingen M, Damaj B, Maghazachi AA. Expression and regulation of chemokine receptors in human natural killer cells. *Blood* 2001;**97**:367–375.

IPD—KIR Database Statistics | EBI. 2019. https://www.ebi.ac.uk/ipd/kir/stats.html (11 September 2020, date last accessed).

Ishitani A, Geraghty DE. *Alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens (mRNA/nonclassical class I antigen/placenta)*. 1992.

Ishitani A, Sageshima N, Lee N, Dorofeeva N, Hatake K, Marquardt H, Geraghty DE. protein expression and peptide binding suggest unique and interacting functional roles for HLA-E, F, and G in maternal-placental immune recognition. *J Immunol* 2003;**171**:1376–1384.

Jablonowska B, Selbing A, Palfi M, Ernerudh J, Kjellberg S, Lindton B. Prevention of recurrent spontaneous abortion by intravenous immunoglobulin: a double-blind placebo-controlled study. *Hum Reprod* 1999;**14**:838–841.

Jacobs R, Hintzen G, Kemper A, Beul K, Kempf S, Behrens G, Sykora KW, Schmidt RE. CD56bright cells differ in their KIR repertoire and cytotoxic features from CD56dim NK cells. *Eur J Immunol* 2001;**31**:3121–3126.

James-Allan LB, Whitley GS, Leslie K, Wallace AE, Cartwright JE. Decidual cell regulation of trophoblast is altered in pregnancies at risk of pre-eclampsia. *J Mol Endocrinol* 2018;**60**:239–246.

Jauniaux E, Hempstock J, Greenwold N, Burton GJ. Trophoblastic oxidative stress in relation to temporal and regional differences in maternal placental blood flow in normal and abnormal early pregnancies. *Am J Pathol* 2003;**162**:115–125.

Johnson PM, Chia KV, Hart CA, Griffith HB, Francis WJA. Trophoblast membrane infusion for unexplained recurrent miscarriage. *BJOG* 1988;**95**:342–347.

Jokhi PP, King A, Sharkey AM, Smith SK, Loke YW. Screening for cytokine messenger ribonucleic acids in purified human decidual lymphocyte populations by the reverse-transcriptase polymerase chain reaction. *J Immunol* 1994;**153**:4427–4435.

Jones RL, Hannan NJ, Kaitu'u TJ, Zhang J, Salamonsen LA. Identification of chemokines important for leukocyte recruitment to the human endometrium at the times of embryo implantation and menstruation. *J Clin Endocrinol Metab* 2004;**89**:6155–6167.

Jonges LE, Albertsson P, Van Vlierberghe RLP, Ensink NG, Johansson BR, Van De Velde CJH, Fleuren GJ, Nannmark U, Kuppen PJK. The phenotypic heterogeneity of human natural killer cells: presence of at least 48 different subsets in the peripheral blood. *Scand J Immunol* 2001;**53**:103–110.

Kam EPY, Gardner L, Loke YW, King A. The role of trophoblast in the physiological change in decidual spiral arteries. *Hum Reprod* 1999;**14**:2131–2138.

Kämmerer U, Rieger L, Kapp M, Dietl J, Ruck P. Immunocompetent cells in the endometrium of fetuses and children. *Hum Reprod* 2003;**18**:969–975.

Keskin DB, Allan DSJ, Rybalov B, Andzelm MM, Stern JNH, Kopcow HD, Koopman LA, Strominger JL. TGFβ promotes conversion of CD16+ peripheral blood NK cells into CD16- NK cells with similarities to decidual NK cells. *Proc Natl Acad Sci U S A* 2007;**104**:3378–3383.

Kharfi A, Giguère Y, Sapin V, Massé J, Dastugue B, Forest JC. Trophoblastic remodeling in normal and preeclamptic pregnancies: implication of cytokines. *Clin Biochem* 2003;**36**:323–331.

Khong TY, Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *BJOG* 1986;**93**:1049–1059.

King A, Balendran N, Wooding P, Carter NP, Loke YW. Cd3– leukocytes present in the human uterus during early placentation: phenotypic and morphologic characterization of the Cd56++ population. *Dev Immunol* 1991;**1**:169–190.

King A, Burrows T, Verma S, Hiby S, Loke YW. Human uterine lymphocytes. *Hum Reprod Update* 1998;**4**:480–485.

King A, Gardner L, Loke YW. Evaluation of oestrogen and progesterone receptor expression in uterine mucosal lymphocytes. *Hum Reprod* 1996;**11**:1079–1082.

King A, Thomas L, Bischof P. Cell culture models of trophoblast II: trophoblast cell lines - a workshop report. In *Placenta*, Vol **21**. Philadelphia: W.B. Saunders Ltd, 2000, S113–S119.

King A, Wellings V, Gardner L, Loke YW. Immunocytochemical characterization of the unusual large granular lymphocytes in human endometrium throughout the menstrual cycle. *Hum Immunol* 1989;**24**:195–205.

King A. Uterine leukocytes and decidualization. *Hum Reprod Update* 2000;**6**:28–36.

Kitaya K, Yamaguchi T, Honjo H. Central role of interleukin-15 in postovulatory recruitment of peripheral blood CD16(-) natural killer cells into human endometrium. *J Clin Endocrinol Metab* 2005; **90**:2932–2940.

Kitaya K, Yasuda J, Nakayama T, Fushiki S, Honjo H. Effect of female sex steroids on human endometrial CD16neg CD56bright natural killer cells. *Fertil Steril* 2003;**79**:730–734.

Kitaya K, Yasuda J, Yagi I, Tada Y, Fushiki S, Honjo H. IL-15 expression at human endometrium and decidua. *Biol Reprod* 2000;**63**: 683–687.

Koller BH, Geraghty DE, Shimizu Y, DeMars R, Orr HT. HLA-E. A novel HLA class I gene expressed in resting T lymphocytes. *J Immunol* 1988;**141**:897–904.

Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, Masch R, Lockwood CJ, Schachter AD, Park PJ *et al*. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med* 2003;**198**:1201–1212.

Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, Demars R. A class I antigen, HLA-G, expressed in human trophoblasts. *Science (80-)* 1990;**248**:220–223.

Lala PK, Chakraborty C. Factors regulating trophoblast migration and invasiveness: possible derangements contributing to pre-eclampsia and fetal injury. *Placenta* 2003;**24**:575–587.

Lash GE, Naruse K, Innes BA, Robson SC, Searle RF, Bulmer JN. Secretion of angiogenic growth factors by villous cytotrophoblast and extravillous trophoblast in early human pregnancy. *Placenta* 2010a;**31**:545–548.

Lash GE, Naruse K, Robson A, Innes BA, Searle RF, Robson SC, Bulmer JN. Interaction between uterine natural killer cells and extravillous trophoblast cells: Effect on cytokine and angiogenic growth factor production. *Hum Reprod* 2011;**26**:2289–2295.

Lash GE, Otun HA, Innes BA, Percival K, Searle RF, Robson SC, Bulmer JN. Regulation of extravillous trophoblast invasion by uterine natural killer cells is dependent on gestational age. *Hum Reprod* 2010b;**25**:1137–1145.

Lash GE, Robson SC, Bulmer JN. Review: Functional role of uterine natural killer (uNK) cells in human early pregnancy decidua. *Placenta* 2010c;**31**:S87–S92.

Lash GE. Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. *J Leukoc Biol* 2006;**80**:572–580.

Lazetic S, Chang C, Houchins JP, Lanier LL, Phillips JH. Human natural killer cell receptors involved in MHC class I recognition are disulfide-linked heterodimers of CD94 and NKG2 subunits. *J Immunol* 1996;**157**:4741–4745.

Lee KA, Koo JJ, Yoon TK, Do BR, Ko JJ, Cha KY. Immunology: immunosuppression by corticosteroid has no effect on the pregnancy rate in routine in-vitro fertilization/embryo transfer patients. *Hum Reprod* 1994;**9**:1832–1835.

Lee N, Goodlett DR, Ishitani A, Marquardt H, Geraghty DE. HLA-E surface expression depends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences. *J Immunol* 1998; **160**:4951–4960.

Lepin EJM, Bastin JM, Allan DSJ, Roncador G, Braud VM, Mason DY, Van Anton Der Merwe P, McMichael AJ, Bell JI, Powis SH *et al*. Functional characterization of HLA-F and binding of HLA-F tetramers to ILT2 and ILT4 receptors. *Eur J Immunol* 2000;**30**: 3552–3561.

Levron Y, Dviri M, Segol I, Yerushalmi GM, Hourvitz A, Orvieto R, Mazaki-Tovi S, Yinon Y. The "immunologic theory" of preeclampsia revisited: a lesson from donor oocyte gestations. *Am J Obstet Gynecol* 2014;**211**:383.e1–383.e5.

Li XF, Charnock-Jones DS, Zhang E, Hiby S, Malik S, Day K, Licence D, Bowen JM, Gardner L, King A *et al*. Angiogenic growth factor messenger ribonucleic acids in uterine natural killer cells. *J Clin Endocrinol Metab* 2001;**86**:1823–1834.

Liu L, Huang J, Li TC, Hong XT, Laird S, Dai YD, Tong XM, Zhu HY, Zhang S. The effect of elevated progesterone levels before oocyte retrieval in women undergoing ovarian stimulation for IVF treatment on the genomic profile of peri-implantation endometrium. *J Reprod Immunol* 2017;**121**:17–25.

Ljunggren HG, Kärre K. In search of the "missing self": MHC molecules and NK cell recognition. *Immunol Today* 1990;**11**:237–244.

Llano M, Lee N, Navarro F, García P, Albar JP, Geraghty DE, López-Botet M. HLA-E-bound peptides influence recognition by inhibitory and triggering CD94/NKG2 receptors: preferential response to an HLA-G-derived nonamer. *Eur J Immunol* 1998;**28**:2854–2863.

Loke Y, King A. *Human Implantation: Cell Biology and Immunology*, Vol. **40**. Cambridge: Cambridge University Press, 1995, 312–313.

Lynch L, Golden-Mason L, Eogan M, O'Herlihy C, O'Farrelly C. Cells with haematopoietic stem cell phenotype in adult human endometrium: Relevance to infertility? *Hum Reprod* 2007;**22**:919–926.

Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N *et al*. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science (80-)* 1997;**277**: 55–60.

Male V, Hughes T, McClory S, Colucci F, Caligiuri MA, Moffett A. Immature NK cells, capable of producing IL-22, are present in human uterine mucosa. *J Immunol* 2010;**185**:3913–3918.

Manaseki S, Searle RF. Natural killer (NK) cell activity of first trimester human decidua. *Cell Immunol* 1989;**121**:166–173.

Manaster I, Mizrahi S, Goldman-Wohl D, Sela HY, Stern-Ginossar N, Lankry D, Gruda R, Hurwitz A, Bdolah Y, Haimov-Kochman R *et al*. Endometrial NK cells are special immature cells that await pregnancy. *J Immunol* 2008;**181**:1869–1876.

Mandelboim O, Reyburn HT, Valés-Gómez M, Pazmany L, Colonna M, Borsellino G, Strominger JL. Protection from lysis by natural killer cells of group 1 and 2 specificity is mediated by residue 80 in human histocompatibility leukocyte antigen C alleles and also

occurs with empty major histocompatibility complex molecules. *J Exp Med* 1996;**184**:913–922.

Martini AE, Jasulaitis S, Fogg LF, Uhler ML, Hirshfeld Cytron JE. Evaluating the utility of intralipid infusion to improve live birth rates in patients with recurrent pregnancy loss or recurrent implantation failure. *J Hum Reprod Sci* 2018;**11**:261–268.

Matsubayashi H, Shida M, Kondo A, Suzuki T, Sugi T, Izumi SI, Hosaka T, Makino T. Preconception peripheral natural killer cell activity as a predictor of pregnancy outcome in patients with unexplained infertility. *Am J Reprod Immunol* 2005;**53**:126–131.

Matsuura-Sawada R, Murakami T, Ozawa Y, Nabeshima H, Akahira JI, Sato Y, Koyanagi Y, Ito M, Terada Y, Okamura K. Reproduction of menstrual changes in transplanted human endometrial tissue in immunodeficient mice. *Hum Reprod* 2005;**20**:1477–1484.

Mavilio D, Lombardo G, Benjamin J, Kim D, Follman D, Marcenaro E, O'Shea MA, Kinter A, Kovacs C, Moretta A *et al.* Characterization of CD56-/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. *Proc Natl Acad Sci U S A* 2005;**102**:2886–2891.

Meng L, Lin J, Chen L, Wang Z, Liu M, Liu Y, Chen X, Zhu L, Chen H, Zhang J. Effectiveness and potential mechanisms of intralipid in treating unexplained recurrent spontaneous abortion. *Arch Gynecol Obstet* 2016;**294**:29–39.

Moffett A, Chazara O, Colucci F, Johnson MH. Variation of maternal KIR and fetal HLA-C genes in reproductive failure: too early for clinical intervention. *Reprod Biomed Online* 2016;**33**:763–769.

Moffett A, Colucci F. Co-evolution of NK receptors and HLA ligands in humans is driven by reproduction. *Immunol Rev* 2015;**267**:283–297.

Moffett A, Colucci F. Uterine NK cells: active regulators at the maternal-fetal interface. *J Clin Invest* 2014;**124**:1872–1879.

Moffett A, Loke C. Immunology of placentation in eutherian mammals. *Nat Rev Immunol* 2006;**6**:584–594.

Moffett A, Shreeve N. First do no harm: Uterine natural killer (NK) cells in assisted reproduction. *Hum Reprod* 2015;**30**:1519–1525.

Moffett-King A. Natural killer cells and pregnancy. *Nat Rev Immunol* 2002;**2**:656–663.

Moffitt D, Queenan JT, Veeck LL, Schoolcraft W, Miller CE, Muasher SJ. Low-dose glucocorticoids after in vitro fertilization and embryo transfer have no significant effect on pregnancy rate. *Fertil Steril* 1995;**63**:571–577.

Moraru M, Carbone J, Alecsandru D, Castillo-Rama M, García-Segovia A, Gil J, Alonso B, Aguarón A, Ramos-Medina R, Martínez de María J *et al.* Intravenous immunoglobulin treatment increased live birth rate in a Spanish cohort of women with recurrent reproductive failure and expanded CD56 + cells. *Am J Reprod Immunol* 2012;**68**:75–84.

Moretta A, Vitale M, Bottino C, Orengo AM, Morelli L, Augugliaro R, Barbaresi M, Ciccone E, Moretta L. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. *J Exp Med* 1993;**178**:597–604.

Moretta L, Moretta A. Killer immunoglobulin-like receptors. *Curr Opin Immunol* 2004;**16**:626–633.

Motteram C, Vollenhoven B, Hope N, Osianlis T, Rombauts LJ. Live birth rates after combined adjuvant therapy in IVF-ICSI cycles: a matched case-control study. *Reprod Biomed Online* 2015;**30**:340–348.

Mowbray JF, Liddell H, Underwood JL, Gibbings C, Reginald PW, Beard RW. Controlled trial of treatment of recurrent spontaneous abortion by immunisation with paternal cells. *Lancet* 1985;**325**:941–943.

Na KH, Lee HJ, Choi JH, Eun JW, Nam SW, Yoon TK, Kim GJ. Dynamic alterations in integrin α4 expression by hypoxia are involved in trophoblast invasion during early implantation. *J Cell Biochem* 2012;**113**:685–694.

Nyborg KM, Kolte AM, Larsen EC, Christiansen OB. Immunomodulatory treatment with intravenous immunoglobulin and prednisone in patients with recurrent miscarriage and implantation failure after in vitro fertilization/intracytoplasmic sperm injection. *Fertil Steril* 2014;**102**:1650–1655.e1.

Ober C, Karrison T, Odem RR, Barnes RB, Branch DW, Stephenson MD, Baron B, Walker MA, Scott JR, Schreiber JR. Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomised trial. *Lancet* 1999;**354**:365–369.

Okada H, Nakajima T, Sanezumi M, Ikuta A, Yasuda K, Kanzaki H. Progesterone enhances interleukin-15 production in human endometrial stromal cells in vitro. *J Clin Endocrinol Metab* 2000;**85**:4765–4770.

Ordi J, Casals G, Ferrer B, Creus M, Guix C, Palacín A, Campo E, Balasch J. Uterine (CD56+) natural killer cells recruitment: association with decidual reaction rather than embryo implantation. *Am J Reprod Immunol* 2006;**55**:369–377.

Ota DM, Jessup JM, Babcock GF, Kirschbaum L, Mountain CF, McMurtrey MJ, Copeland EM. Immune function during intravenous administration of a soybean oil emulsion. *JPEN J Parenter Enteral Nutr* 1985;**9**:23–27.

Pace D, Morrison L, Bulmer JN. Proliferative activity in endometrial stromal granulocytes throughout menstrual cycle and early pregnancy. *J Clin Pathol* 1989;**42**:35–39.

Pandey MK, Agrawal S. Induction of MLR-Bf and protection of fetal loss: a current double blind randomized trial of paternal lymphocyte immunization for women with recurrent spontaneous abortion. *Int Immunopharmacol* 2004;**4**:289–298.

Papuchova H, Kshirsagar S, Xu L, Gomes HAB, Li Q, Iyer V, Norwitz ER, Strominger JL, Tilburgs T. Three types of HLA-G+ extravillous trophoblasts that have distinct immune regulatory properties. *Proc Natl Acad Sci U S A* 2020;**117**:15772–15777.

Parham P. NK cells and trophoblasts: partners in pregnancy. *J Exp Med* 2004;**200**:951–955.

Pecks U, Maass N, Neulen J. Oocyte donation: a risk factor for pregnancy-induced hypertension a meta-analysis and case series. *Dtsch Arztebl Int* 2011;**108**:23–31.

Peel S. *Granulated Metrial Gland Cells*. Advances in Anatomy Embryology and Cell Biology. Berlin: Springer-Verlag. 1989;**115**:1–26.

Pegoraro E, Whitaker J, Mowery-Rushton P, Surti U, Lanasa M, Hoffman EP. Familial skewed X inactivation: a molecular trait associated with high spontaneous-abortion rate maps to Xq28. *Am J Hum Genet* 1997;**61**:160–170.

Perino A, Vassiliadis A, Vucetich A, Colacurci N, Menato G, Cignitti M, Semprini AE. Short-term therapy for recurrent abortion using

intravenous immunoglobulins: results of a double-blind placebo-controlled Italian study. *Hum Reprod* 1997;**12**:2388–2392.

Pijnenborg R, Vercruysse L, Hanssens M, Van Assche FA. Incomplete trophoblast invasion: the evidence. *Preeclampsia* 2003, RCOG Press,15–26.

Pijnenborg R, Bland JM, Robertson WB, Brosens I. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. *Placenta* 1983;**4**:397–413.

Plaçais L, Kolanska K, Kraiem YB, Cohen J, Suner L, Bornes M, Sedille L, Rosefort A, D'Argent EM, Selleret L *et al*. Intralipid therapy for unexplained recurrent miscarriage and implantation failure: case-series and literature review. *Eur J Obstet Gynecol Reprod Biol* 2020;**252**:100–104.

Placido GD, Zullo F, Mollo A, Cappiello F, Nazzaro A, Colacurci N, Palumbo G. Intravenous immunoglobulin (IVIG) in the prevention of implantation failures. *Ann NY Acad Sci* 1994;**734**:232–234.

Polanski LT, Barbosa MAP, Martins WP, Baumgarten MN, Campbell B, Brosens J, Quenby S, Raine-Fenning N. Interventions to improve reproductive outcomes in women with elevated natural killer cells undergoing assisted reproduction techniques: a systematic review of literature. *Hum Reprod* 2014;**29**:65–75.

Ponte M, Cantoni C, Biassoni R, Tradori-Cappai A, Bentivoglio G, Vitale C, Bertone S, Moretta A, Moretta L, Mingari MC. Inhibitory receptors sensing HLA-G1 molecules in pregnancy: decidua-associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, an HLA-G1-specific receptor. *Proc Natl Acad Sci U S A* 1999;**96**:5674–5679.

Puzanov IJ, Bennett M, Kumar V. IL-15 can substitute for the marrow microenvironment in the differentiation of natural killer cells. *J Immunol* 1996;**157**:4282–4285.

Quenby S, Bates M, Doig T, Brewster J, Lewis-Jones DI, Johnson PM, Vince G. Pre-implantation endometrial leukocytes in women with recurrent miscarriage. *Hum Reprod* 1999;**14**:2386–2391.

Quenby S, Kalumbi C, Bates M, Farquharson R, Vince G. Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. *Fertil Steril* 2005;**84**: 980–984.

Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J, Bulmer J. Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod* 2008;**24**:45–54.

Quenby S, Vince G, Farquharson R, Aplin J. *Recurrent miscarriage: a defect in nature's quality control?* 2002.

Rajagopalan S, Long EO. A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. *J Exp Med* 1999;**189**:1093–1099.

Rajalingam R, Du Z, Meenagh A, Luo L, Kavitha VJ, Pavithra-Arulvani R, Vidhyalakshmi A, Sharma SK, Balazs I, Reed EF *et al*. Distinct diversity of KIR genes in three southern Indian populations: comparison with world populations revealed a link between KIR gene content and pre-historic human migrations. *Immunogenetics* 2008; **60**:207–217.

Red-Horse K, Drake PM, Gunn MD, Fisher SJ. Chemokine ligand and receptor expression in the pregnant uterus: Reciprocal patterns in complementary cell subsets suggest functional roles. *Am J Pathol* 2001;**159**:2199–2213.

Revelli A, Dolfin E, Gennarelli G, Lantieri T, Massobrio M, Holte JG, Tur-Kaspa I. Tur-Kaspa I. Low-dose acetylsalicylic acid plus prednisolone as an adjuvant treatment in IVF: a prospective, randomized study. *Fertil Steril* 2008;**90**:1685–1691.

Ritson A, Bulmer JN. Endometrial granulocytes in human decidua react with a natural-killer (NK) cell marker, NKH1. *Immunology* 1987;**62**:329–331.

Roberts JM. *Pre-Eclampsia: A Two-Stage Disorder*. London: RCOG Press, 2003, 66–78.

Robson A, Harris LK, Innes BA, Lash GE, Aljunaidy MM, Aplin JD, Baker PN, Robson SC, Bulmer JN. Uterine natural killer cells initiate spiral artery remodeling in human pregnancy. *FASEB J* 2012;**26**: 4876–4885.

Robson A, Lash GE, Innes BA, Zhang JY, Robson SC, Bulmer JN. Uterine spiral artery muscle dedifferentiation. *Hum Reprod* 2019; **34**:1428–1438.

Rodesch F, Simon P, Donner C, Jauniaux E. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstet Gynecol* 1992;**80**:283–285.

Rokhafrooz S, Ghadiri A, Ghandil P, Ghafourian M, Hossaini SH, Daraei N, Najafian M, Rouhizadeh A. Association between HLA-G 14bp gene polymorphism and serum sHLA-G protein concentrations in preeclamptic patients and normal pregnant women. *Immunol Invest* 2018;**47**:558–568.

Rossant J, Cross JC. Placental development: lessons from mouse mutants. *Nat Rev Genet* 2001;**2**:538–548.

Roth I, Fisher SJ. IL-10 is an autocrine inhibitor of human placental cytotrophoblast MMP- 9 production and invasion. *Dev Biol* 1999; **205**:194–204.

Ruiz JE, Kwak JYH, Baum L, Gilman-Sachs A, Beaman KD, Kim YB, Beer AE. Intravenous immunoglobulin inhibits natural killer cell activity in vivo in women with recurrent spontaneous abortion. *Am J Reprod Immunol* 1996;**35**:370–375.

Saito S, Nishikawa K, Morii T, Enomoto M, Narita N, Motoyoshi K, Ichijo M. Cytokine production by CD16-CD56bright natural killer cells in the human early pregnancy decidua. *Int Immunol* 1993;**5**:559–563.

Saito S, Takeda Y, Sakai M, Nakabayahi M, Hayakawa S. The incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men. *J Reprod Immunol* 2006;**70**:93–98.

Samaridis J, Colonna M. Cloning of novel immunoglobulin superfamily receptors expressed on human myeloid and lymphoid cells: Structural evidence for new stimulatory and inhibitory pathways. *Eur J Immunol* 1997;**27**:660–665.

Santjohanser C, Knieper C, Franz C, Hirv K, Meri O, Schleyer M, Würfel W, Toth B. Granulocyte-colony stimulating factor as treatment option in patients with recurrent miscarriage. *Arch Immunol Ther Exp (Warsz)* 2013;**61**:159–164.

Santoni A, Carlino C, Stabile H, Gismondi A. Mechanisms underlying recruitment and accumulation of decidual NK cells in uterus during pregnancy. *Am J Reprod Immunol* 2008;**59**:417–424.

Scaradavou A. HLA antigens. In: *Transfusion Medicine and Hemostasis: Clinical and Laboratory Aspects*, 2nd ed. Amsterdam: Elsevier Inc, 2013, 191–197.

Scarpellini F, Sbracia M. Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: a randomised controlled trial. *Hum Reprod* 2009;**24**:2703–2708.

Scott J. Immunotherapy for recurrent miscarriage. In: *Cochrane Database of Systematic Reviews*. Hoboken: John Wiley & Sons, Ltd, 2003.

Sentman CL, Meadows SK, Wira CR, Eriksson M. Recruitment of uterine NK cells: induction of CXC chemokine ligands 10 and 11 in human endometrium by estradiol and progesterone. *J Immunol* 2004;**173**:6760–6766.

Sharkey AM, Gardner L, Hiby S, Farrell L, Apps R, Masters L, Goodridge J, Lathbury L, Stewart CA, Verma S *et al*. Killer Ig-like receptor expression in uterine NK cells is biased toward recognition of HLA-C and alters with gestational age. *J Immunol* 2008; **181**:39–46.

Shehata H. Baby born to woman who suffered 20 miscarriages. *BBC News*. 2014. https://www.bbc.com/news/health-25775823 (10 April 2020, date last accessed).

Shivhare SB, Bulmer JN, Innes BA, Hapangama DK, Lash GE. Altered vascular smooth muscle cell differentiation in the endometrial vasculature in menorrhagia. *Hum Reprod* 2014;**29**:1884–1894.

Shobu T, Sageshima N, Tokui H, Omura M, Saito K, Nagatsuka Y, Nakanishi M, Hayashi Y, Hatake K, Ishitani A. The surface expression of HLA-F on decidual trophoblasts increases from mid to term gestation. *J Reprod Immunol* 2006;**72**:18–32.

Shreeve N, Sadek K. Intralipid therapy for recurrent implantation failure: new hope or false dawn? *J Reprod Immunol* 2012;**93**:38–40.

Siristatidis C, Dafopoulos K, El-Khayat W, Salamalekis G, Anifandis G, Vrantza T, Elsadek M, Papantoniou N. Administration of prednisolone and low molecular weight heparin in patients with repeated implantation failures: a cohort study. *Gynecol Endocrinol* 2018;**34**:136–139.

Smith SD, Dunk CE, Aplin JD, Harris LK, Jones RL. Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. *Am J Pathol* 2009;**174**:1959–1971.

Sojka DK, Yang L, Plougastel-Douglas B, Higuchi DA, Croy BA, Yokoyama WM. Cutting edge: local proliferation of uterine tissue-resident NK cells during decidualization in mice. *J Immunol* 2018; **201**:2551–2556.

Sojka DK, Yang L, Yokoyama WM. Uterine natural killer cells. *Front Immunol* 2019;**10**:960.

Spandidos DA, Koumantakis E, Sifakis S, Sourvinos G. Microsatellite mutations in spontaneously aborted embryos. *Fertil Steril* 1998;**70**: 892–895.

Spornitz UM. The functional morphology of the human endometrium and decidua. *Adv Anat Embryol Cell Biol* 1992;**124**:1–99.

Starkey PM, Sargent IL, Redman CW. Cell populations in human early pregnancy decidua: characterization and isolation of large granular lymphocytes by flow cytometry. *Immunology* 1988;**65**: 129–134.

Steinborn A, Varkonyi T, Scharf A, Bahlmann F, Klee A, Sohn C. Early detection of decreased soluble HLA-G levels in the maternal circulation predicts the occurrence of preeclampsia and intrauterine growth retardation during further course of pregnancy. *Am J Reprod Immunol* 2007;**57**:277–286.

Stephenson MD, Dreher K, Houlihan E, Wu V. Prevention of unexplained recurrent spontaneous abortion using intravenous immunoglobulin: a prospective, randomized, double-blinded, placebo-controlled trial. *Am J Reprod Immunol* 1998;**39**:82–88.

Stephenson MD, Kutteh WH, Purkiss S, Librach C, Schultz P, Houlihan E, Liao C. Intravenous immunoglobulin and idiopathic secondary recurrent miscarriage: a multicentered randomized placebo-controlled trial. *Hum Reprod* 2010;**25**:2203–2209.

Stewart CA, Laugier-Anfossi F, Vely F, Saulquin X, Riedmuller J, Tisserant A, Gauthier L, Romagne F, Ferracci G, Arosa FA *et al*. Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors. *Proc Natl Acad Sci U S A* 2005;**102**: 13224–13229.

Su MT, Lin SH, Chen YC, Kuo PL. Genetic association studies of ACE and PAI-1 genes in women with recurrent pregnancy loss: a systematic review and meta-analysis. *Thromb Haemost* 2013;**109**: 8–15.

Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *J Am Med Assoc* 2004;**292**:81–85.

Tempfer C, Unfried G, Zeillinger R, Hefler L, Nagele F, Huber JC. Endothelial nitric oxide synthase gene polymorphism in women with idiopathic recurrent miscarriage. *Hum Reprod* 2001;**16**: 1644–1647.

Tempfer CB, Kurz C, Bentz EK, Unfried G, Walch K, Czizek U, Huber JC. A combination treatment of prednisone, aspirin, folate, and progesterone in women with idiopathic recurrent miscarriage: a matched-pair study. *Fertil Steril* 2006;**86**:145–148.

Thaler I, Manor D, Itskovitz J, Rottem S, Levit N, Timor-Tritsch I, Brandes JM. Changes in uterine blood flow during human pregnancy. *Am J Obstet Gynecol* 1990;**162**:121–125.

Trundley A, Moffett A. Human uterine leukocytes and pregnancy. *Tissue Antigens* 2004;**63**:1–12.

Tsai AF, Kaufman KA, Walker MA, Karrison TG, Odem RR, Barnes RB, Scott JR, Schreiber JR, Stephenson MD, Ober C. Transmission disequilibrium of maternally-inherited CTLA-4 microsatellite alleles in idiopathic recurrent miscarriage. *J Reprod Immunol* 1998;**40**: 147–157.

Tuckerman E, Laird SM, Prakash A, Li TC. Prognostic value of the measurement of uterine natural killer cells in the endometrium of women with recurrent miscarriage. *Hum Reprod* 2007;**22**: 2208–2213.

Ubaldi F, Rienzi L, Ferrero S, Anniballo R, Iacobelli M, Cobellis L, Greco E. Low dose prednisolone administration in routine ICSI patients does not improve pregnancy and implantation rates. *Hum Reprod* 2002;**17**:1544–1547.

Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, Tyan D, Lanier LL, Parham P. Human diversity in killer cell inhibitory receptor genes. *Immunity* 1997;**7**:753–763.

Ulbrecht M, Honka T, Person S, Johnson JP, Weiss EH. The HLA-E gene encodes two differentially regulated transcripts and a cell surface protein. *J Immunol* 1992;**149**:2945–2953.

Vacca P, Moretta L, Moretta A, Mingari MC. Origin, phenotype and function of human natural killer cells in pregnancy. *Trends Immunol* 2011;**32**:517–523.

van der Meer A, Lukassen HGM, van Lierop MJC, Wijnands F, Mosselman S, Braat DDM, Joosten I. Membrane-bound HLA-G activates proliferation and interferon-γ production by uterine natural killer cells. *Mol Hum Reprod* 2004;**10**:189–195.

Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, Park JE, Stephenson E, Polański K, Goncalves A *et al*. Single-cell reconstruction of the early maternal–fetal interface in humans. *Nature* 2018;**563**:347–353.

Verma S, Hiby SE, Loke YW, King A. Human Decidual Natural Killer Cells Express the Receptor for and Respond to the Cytokine Interleukin 151. *Biol Reprod* 2000;**62**:959–968.

Verma S, King A, Loke YW. Expression of killer cell inhibitory receptors on human uterine natural killer cells. *Eur J Immunol* 1997;**27**: 979–983.

Vilches C, Parham P. KIR: Diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu Rev Immunol* 2002;**20**:217–251.

Wagtmann N, Rajagopalan S, Winter CC, Peruui M, Long EO. Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer. *Immunity* 1995;**3**: 801–809.

Wagtmann N, Rojo S, Eichler E, Mohrenweiser H, Long EO. A new human gene complex encoding the killer cell inhibitory receptors and related monocyte/macrophage receptors. *Curr Biol* 1997;**7**: 615–618.

Wallace AE, Whitley GS, Thilaganathan B, Cartwright JE. Decidual natural killer cell receptor expression is altered in pregnancies with impaired vascular remodeling and a higher risk of pre-eclampsia. *J Leukoc Biol* 2015;**97**:79–86.

Wang Q, Li TC, Wu YP, Cocksedge KA, Fu YS, Kong QY, Yao SZ. Reappraisal of peripheral NK cells in women with recurrent miscarriage. *Reprod Biomed Online* 2008;**17**:814–819.

Warren B. Blaustein's Pathology of the Female Genital Tract. *Pathology* 1995;**27**:109– 633. 1111–1113.

Wei X, Orr HT. Differential expression of HLA-E, HLA-F, and HLA-G transcripts in human tissue. *Hum Immunol* 1990;**29**:131–142.

Weill P. Etudes sur les leucocytes. I. Les cellules granuleuses des muqueses intestinale et uterine. *Arch D'Anatomie Microsc* 1921;

Whitelaw PF, Croy BA. Granulated lymphocytes of pregnancy. *Placenta* 1996;**17**:533–543.

Wilkens J, Male V, Ghazal P, Forster T, Gibson DA, Williams ARW, Brito-Mutunayagam SL, Craigon M, Lourenco P, Cameron IT *et al.* Uterine NK Cells Regulate Endometrial Bleeding in Women and Are Suppressed by the Progesterone Receptor Modulator Asoprisnil. *J Immunol* 2013;**191**:2226–2235.

Williams PJ, Searle RF, Robson SC, Innes BA, Bulmer JN. Decidual leucocyte populations in early to late gestation normal human pregnancy. *J Reprod Immunol* 2009;**82**:24–31.

Winger EE, Reed JL, Ashoush S, Ahuja S, El-Toukhy T, Taranissi M. Treatment with Adalimumab (Humira®) and intravenous immunoglobulin improves pregnancy rates in women undergoing IVF. *Am J Reprod Immunol* 2008;**61**:113–120.

Winger EE, Reed JL, Ashoush S, El-Toukhy T, Ahuja S, Taranissi M. Elevated preconception CD56 +16 + and/or Th1:Th2 levels predict benefit from IVIG therapy in subfertile women undergoing IVF. *Am J Reprod Immunol* 2011;**66**:394–403.

Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. *J Immunol* 1998; **161**:571–577.

Wong LF, Porter TF, Scott JR. Immunotherapy for recurrent miscarriage. *Cochrane Database Syst Rev* 2014;**2014**:CD00112.

Woon EV, Day A, Bracewell-Milnes T, Male V, Johnson M. Immunotherapy to improve pregnancy outcome in women with abnormal natural killer cell levels/activity and recurrent miscarriage or implantation failure: a systematic review and meta-analysis. *J Reprod Immunol* 2020;**142**:103189.

Xiong S, Sharkey AM, Kennedy PR, Gardner L, Farrell LE, Chazara O, Bauer J, Hiby SE, Colucci F, Moffett A. Maternal uterine NK cell-activating receptor KIR2DS1 enhances placentation. *J Clin Invest* 2013;**123**:4264–4272.

Yamada H, Morikawa M, Kato EH, Shimada S, Kobashi G, Minakami H. Pre-conceptional natural killer cell activity and percentage as predictors of biochemical pregnancy and spontaneous abortion with normal chromosome karyotype. *Am J Reprod Immunol* 2003; **50**:351–354.

Yang HW, Hwang KJ, Kwon HC, Kim HS, Choi KW, Oh KS. Detection of reactive oxygen species (ROS) and apoptosis in human fragmented embryos. *Hum Reprod* 1998;**13**:998–1002.

Yedwab GA, Paz G, Homonnai TZ, David MP, Kraicer PF. The temperature, pH, and partial pressure of oxygen in the cervix and uterus of women and uterus of rats during the cycle. *Fertil Steril* 1976;**27**:304–309.

Yie SM, Taylor RN, Librach C. Low plasma HLA-G protein concentrations in early gestation indicate the development of preeclampsia later in pregnancy. *Am J Obstet Gynecol* 2005;**193**:204–208.

Yokoyama WM. Recognition structures on natural killer cells. *Curr Opin Immunol* 1993;**5**:67–73.

Zafardoust S, Akhondi MM, Sadeghi MR, Mohammadzadeh A, Karimi A, Jouhari S, Ansaripour S. Efficacy of intrauterine injection of granulocyte colony stimulating factor (G-CSF) on treatment of unexplained recurrent miscarriage: a pilot RCT study. *J Reprod Infertil* 2017;**18**:379–385.