Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis

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INTRODUCTION: Embryo implantation is a complex process involving maternal hormonal changes, immune responses and maturational events in the embryo. A pregnancy could fail when these events are not synchronized. It is speculated that in women, an elevation of natural killer (NK) cells may have an effect on reproductive performance, and NK cell levels in blood are currently being used as a diagnostic test to guide the initiation of therapies in patients with infertility.

METHODS: We conducted a systematic review to evaluate the (i) levels of NK cells in blood and endometrium in infertile versus fertile women, (ii) association between NK cells and IVF outcome, (iii) levels of NK cells in blood and endometrium in women with recurrent miscarriage (RM) versus controls. The following electronic databases were searched: Medline, EMBASE, Cochrane Library, Web of Science and National Research Register.

RESULTS: A total of 22 studies were included. Meta-analysis of studies that evaluated peripheral and uterine NK (uNK) cell percentages in infertile versus fertile women showed no significant difference between the two groups [standardized mean difference (SMD) —0.33; 95% confidence intervals (CI) —1.06, 0.4; P = 0.37; SMD —1.82; 95% CI —4.80, 1.17; P = 0.23 respectively]. Pooling of studies that reported peripheral NK cells as numbers showed significantly higher NK cell numbers in infertile women compared with fertile controls (SMD 3.16; 95% CI 1.07, 5.24; P = 0.003). Meta-analysis of studies that evaluated the role of NK cells in IVF outcome showed no significant difference in live birth rates in women with elevated peripheral NK cells or NK cell activity compared with women without elevated peripheral NK cells or NK cell activity (NK activity assessed using a cytotoxicity assay) (relative risk 0.57; 95% CI 0.06, 5.22; P = 0.62). Meta-analysis of studies that evaluated peripheral NK cell percentages in women with RM versus controls showed significantly higher NK cell percentages in women with RM (SMD 1.36; 95% CI 0.04, 2.69; P = 0.03). Meta-analysis of studies that evaluated peripheral NK cell numbers showed significantly higher NK cell numbers in women with RM compared with controls (SMD 0.81; 95% CI 0.47, 1.16; P < 0.0001). Meta-analysis of studies that evaluated uNK cells showed no significant difference in women with RM compared with controls (SMD 0.40; 95% CI —1.24, 2.04; P = 0.63).

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**CONCLUSIONS:** Further research is needed before NK cell assessment can be recommended as a diagnostic tool in the context of female infertility or RM. There is no clear explanation as to why the results differ when data for NK cells are expressed as numbers or a percentage. On the basis of current evidence, NK cell analysis and immune therapy should be offered only in the context of clinical research.

**Key words:** natural killer cells / infertility / IVF / recurrent miscarriage

## Introduction

Embryo implantation is influenced by local and systemic immune responses involving immunoglobulins, cytokines, hormonal and other endometrial factors. A synergism of these factors is critical for successful implantation and subsequent conception. Natural killer (NK) cells have been implicated to play a role in female reproductive performance (Beer et al., 1996; Quenby et al., 1999). They have been thought to be associated with implantation failures, recurrent miscarriage (RM) or infertility due to either NK cell cytotoxicity or receptor/gene expression (Kwak-Kim and Gilman-Sachs, 2008). NK cells are a type of large granular lymphocyte that belong to the innate immune system. They are derived from haematopoietic progenitor cells (HPCs) in the bone marrow and express the surface marker CD 56 (Robertson and Ritz, 1990). The HPCs are activated through different stages of maturation to produce CD 56 bright or dim NK cells. NK cells cause cytoxic effects by inducing lysis or apoptosis of the target cells mediated by the release of granular components within their cytoplasm (perforin, granzymes) or secretion of cytokines, such as tumour necrosis factor-alpha, interleukin (IL)-10, interferon-gamma and transforming growth factor-beta. NK cells have been reported as either as numbers, percentage of the total circulating lymphocytes or of the total stromal endometrial cells. Peripheral NK cells are measured in the blood by flow cytometry. Uterine NK (uNK) cells are evaluated using monoclonal antibodies by immunohistochemical staining of tissue or by flow cytometry of cells obtained by endometrial biopsy. There is no consensus in the published literature with regard to the timing for peripheral NK cell testing. There is also a variation in peripheral NK cell levels when tested during the different phases of the menstrual cycle (Sulke et al., 1985). Although uNK cells were mostly measured in the late luteal phase in the studies, there is cycle-to-cycle variation in the number of uNK cells (Mariee et al., 2012). NK cell testing in the literature has included the measurement of: (i) NK cell subsets identified by cell surface density (CD) of CD 56, (ii) antibody-dependent cellular cytotoxicity or natural cytotoxicity mediated by cytokines produced by CD 56 dim cells (CD 56 dim/16+), (iii) CD 56 bright and CD 16 dim/neg NK cells in peripheral blood which are thought to be an important inflammatory or regulatory subset, (iv) CD 69, a specific activation marker which induces the release of cytokines and the further activation of NK cells (Marzio et al., 1999), (v) other NK cell subsets (NK3/NK1 cells), (vi) NK cell receptor expression (CD 94, CD 128, CD 122, CD 25, CD 30, CD 154 and receptor for IL-2) and (vii) killer cell immunoglobulin like receptors expression on peripheral/uNK cells (Fig. 1).

NK cell subsets (CD 56 dim, CD 56 bright) have differing cytotoxicity, secretory cytokine profile and receptor/gene expression. The majority (90%) of peripheral NK cells are CD 56 dim and express high levels of CD 16 (Kwak-Kim and Gilman-Sachs, 2008). uNK cells are phenotypically and functionally different from peripheral NK cells (Moller et al., 1998). Most uNK cells are characterized by the presence of CD 56 antigen and the absence of the CD 16 antigen (Bulmer and Lash, 2005), whereas the majority of peripheral NK cells express both CD 56 and CD 16. uNK cells are understood to be the most important leucocytes in the preimplantation endometrium and early pregnancy decidua, responsible for trophoblast invasion (Lash et al., 2006). uNK cells have an immunoregulatory potential that peripheral NK cells do not demonstrate (Koopman et al., 2003). As a result, data derived from the peripheral NK cells in infertile women and women with RM have to be interpreted with caution and may not represent what happens at the feto-maternal interface. However, there is emerging evidence that uNK cells arise from the trafficking of peripheral NK cells into the uterus and their subsequent differentiation (Kitaya et al., 2005, 2007; Anne Cray et al., 2006).

The relationship between NK cells and reproductive outcome is one of the most controversial areas in reproductive medicine. The prevalence of CD 56 +ve NK cells in the blood is ~10% of the total peripheral blood lymphocytes (Robertson and Ritz, 1990). Prospective observational and case-control studies have reported that levels of CD 56 +ve NK cells ≥12% are associated with poor reproductive outcomes (Michou et al., 2003; Thum et al., 2004). Studies are contradictory in reporting the association between NK cells and IVF outcome (Beer et al., 1996; Thum et al., 2004; Baczkowski and Kurzawa, 2007). A recent study has reported an NK percentage >18% as being highly specific for women with RM and women with recurrent implantation failure (King et al., 2010). NK cells have also been implicated in the etiopathogenesis of RM (Fukui et al., 2008, 2011).

Despite the contradictory data, peripheral NK cell testing is being promoted as a useful diagnostic test to guide the initiation of a variety of immunosuppressive therapies amongst patients with infertility (Rai et al., 2005). Owing to the wide variety of NK cell testing being offered to infertile women and women with a history of RM, we aimed to establish the prevalence of NK cells in these groups. We also explored a role of raised NK cell levels in IVF outcome. We therefore conducted a systematic review of the literature to evaluate (i) the levels of NK cells in peripheral blood and endometrium in infertile versus fertile women, (ii) the association between NK cells and IVF outcome and (iii) the levels of NK cells in peripheral blood and endometrium women with RM versus controls. We also attempted to explore the methodological and technical inconsistencies in the published literature.

## Methods

### Identification of literature

The following electronic databases were searched: MEDLINE (1950 to April 2012), EMBASE (1980 to April 2012), Cochrane Library, Central register of Controlled Trials and Web of Science (1990 to April 2012). A combination of Medical Subject Headings (MeSH) and text words were used to generate subsets of citations and individual subsets were combined to generate a
single set of citations for each of the three questions. Studies of NK cells (‘NK cells’, ‘CD 56’, ‘CD 69’, ‘T Lymphocytes’, ‘T suppressor cells’) AND infertility (‘infertility’, ‘subfertility’, ‘fertility’) were combined to address the first question (see above), studies of NK cells and IVF (‘IVF’, ‘in vitro fertilization’, ‘fertilization-in vitro’, ‘intracytoplasmic sperm injection’, ‘sperm injection intracytoplasmic’, ‘reproductive techniques assisted’, ‘embryo transfer’ and ‘embryo implantation’) to address the second question and studies NK cells AND RM (‘recurrent miscarriage’, ‘recurrent abortion’, ‘recurrent pregnancy loss’) to address the third question. The reference lists of all known primary and review articles were examined to identify cited articles not captured by electronic searches. Articles which were frequently quoted were used in the science citation index to identify additional citations. No language restrictions were placed in any of our searches. The searches were conducted independently by S.S. and S.K.S.

Study selection and data extraction

The inclusion criterion was as follows: studies evaluating the levels of NK cells in the infertile and fertile population were included to address the first question (see above). Studies were selected for the second question if the target population were women undergoing IVF treatment with measurement of NK cells. Studies that evaluated the prevalence of NK cells in women with RM versus fertile controls were included to address the third question. Studies were selected in a two-stage process. First, the titles and the abstracts from the electronic searches were scrutinized by two reviewers independently (S.S. and S.K.S.) and full manuscripts of all citations that were likely to meet the predefined selection criteria were obtained. Secondly, final inclusion or exclusion decisions were made on examination of the full manuscripts. In the case of duplicates, the most recent or the most comprehensive publication with all the results was used. From each study, outcome
data were extracted in 2 × 2 tables by the two reviewers (S.K.S and S.S.) and two reviewers completed the data extraction and quality assessment (Berlin and Rennie, 1999). The authors of the primary studies were contacted for any missing or unclear information. The Newcastle–Ottawa scale was implemented for quality assessment of observational studies (Wells et al., 2000). Items assessed included selection of cases/cohorts and controls, comparability of the study group and exposure and treatment outcome (Supplementary data, Table S1). The score ranged from 0 to 9, with a score of either 0 or 1 for each item.

**Statistical analysis**

Relative risks (RRs) from individual studies were meta-analysed using a fixed effects model (Mantel and Haenszel, 1959) and random effects model as appropriate (DerSimonian and Laird, 1986). The prevalence of NK cells in the different comparison groups was analysed using standardised mean difference (SMD). Heterogeneity of the exposure effects was evaluated graphically using forest plots (Lewis and Clarke, 2001) and statistically using the I² statistic to quantify heterogeneity across studies (Higgins and Thompson, 2002). We explored the causes of heterogeneity using the variation of features of population, exposure and study quality. Statistical analyses were performed using Revman 4.2 and 5 (Cochrane collaboration, Oxford, UK).

**Results**

The search strategy yielded 1110 citations. Of these, 1075 citations were excluded initially as it was clear from the title and abstract that they did not fulfil the selection criteria. Full manuscripts were obtained for the remaining 35 articles for detailed evaluation and following scrutiny, 13 studies were excluded. Three studies were excluded as they were reviews (Moffet et al., 2004; Kwak-Kim et al., 2005; Rai et al., 2005). Five studies were excluded for question one evaluating NK cell levels in infertile women versus controls for the following reasons: three studies had insufficient data for analysis (Lukassen et al., 2004; Baczkowski and Kurzawa, 2007; Lynch et al., 2007) and two studies measured various markers of NK cells (Matsubayashi et al., 2001; Ntrivalas et al., 2001). For the second question addressing the association between NK cells and IVF outcome one study was excluded as the data were replicated (Thum et al., 2004). For the third question evaluating NK cell levels in women with RM versus controls one study was excluded due to inappropriate control group (Fukui et al., 1999), two studies were of inappropriate study design for inclusion (Emmer et al., 2000; Quenby et al., 2005) and one study was excluded due to insufficient data (Perricone et al., 2006). The final inclusion was therefore 22 studies that met the selection criteria for the different questions addressed in the review (Fig. 2). Of the 22 included studies, some studies addressed more than one question set out in this review.

Ten studies evaluated NK cell levels in infertile women versus fertile controls. Nine out of the 10 studies evaluated peripheral NK cells (Opsahl et al., 1994; Fornari et al., 2002; Lukassen et al., 2003; Michou et al., 2003; Vujisic et al., 2004; Ntrivalas et al., 2005; van den Heuvel et al., 2007; McGrath et al., 2009; Sacks et al., 2012) and two studies evaluated uNK cells (McGrath et al., 2009; Parkin et al., 2011). One study evaluated both peripheral and uNK cells (McGrath et al., 2009). Six of the nine studies expressed NK cell levels as percentages (Opsahl et al., 1994; Lukassen et al., 2003; Vujisic et al., 2004; Ntrivalas et al., 2005; McGrath et al., 2009; Sacks et al., 2012) and three studies expressed NK cell levels as numbers (Fornari et al., 2002; Michou et al., 2003; van den Heuvel et al., 2007).

Two studies reported the association between NK cells and IVF outcome (Coulam and Roussev, 2003; Thum et al., 2005). Thirteen studies evaluated peripheral NK cell levels in women with RM versus controls (Aoki et al., 1995; Lachapelle et al., 1996; Clifford et al., 1999; Quenby et al., 1999; Michimata et al., 2002; Michou et al., 2003; Shakhar et al., 2003; Shimada et al., 2004; Ntrivalas et al., 2005; Tuckerman et al., 2007; Wang et al., 2008; King et al., 2010; Parkin et al., 2011). Of these 13 studies, 6 studies evaluated peripheral NK cells (Aoki et al., 1995; Michou et al., 2003) and four studies as percentages (Shakhar et al., 2003; Ntrivalas et al., 2005; Wang et al., 2008; King et al., 2010). Three studies evaluated the levels of NK cells in both infertile women versus controls and women with RM versus controls (Michou et al., 2003; Ntrivalas et al., 2005; Parkin et al., 2011).

The quality assessment of the observational studies is presented in Supplementary data, Table S1. The observational studies scored well on the Newcastle–Ottawa scale; two studies scored eight, 17 studies scored seven and three studies scored six. Of the 22 observational studies, ten were cohort controlled studies (Opsahl et al., 1994; Aoki et al., 1995; Lachapelle et al., 1996; Clifford et al., 1999; Fukui et al., 1999; Quenby et al., 1999; Michimata et al., 2002; Shakhar et al., 2003; Shimada et al., 2004; Tuckerman et al., 2007) and the remaining 12 studies were case control studies (Fornari et al., 2002; Coulam and Roussev, 2003; Michou et al., 2003; Lukassen et al., 2003; Ntrivalas et al., 2005; Thum et al., 2005; van den Heuvel et al., 2007; Wang et al., 2008; McGrath et al., 2009; King et al., 2010; Parkin et al., 2011; Sacks et al., 2012). Five studies were retrospective and the remaining 17 studies were of prospective study design. The main characteristics of all the 22 studies are presented in Supplementary data, Tables SII – SIV. No evidence of publication bias or related biases was suggested from the funnel plot analysis (Begg’s test, P = 0.51, Fig. 3).

**NK cells in infertile women versus fertile women**

Meta-analysis of the six studies that evaluated peripheral NK cell levels as percentages in infertile versus fertile women showed no significant difference between the two groups [SMD = 0.33; 95% confidence interval (CI) = 1.06; 0.40; P = 0.37; Fig. 4a]. Meta-analysis of the three studies that evaluated peripheral NK cells as numbers showed significantly higher levels of peripheral NK cells in infertile compared with fertile women (SMD 3.16; 95% CI 1.07; 5.24; P = 0.003; Fig. 4b). Meta-analysis of the two studies that evaluated uNK cells expressed as percentages in infertile women versus fertile controls showed no significant difference between the two groups (SMD = 1.82; 95%CI = 4.80; 1.17; P = 0.23; Fig. 4c). The I² values were 85, 92 and 95% indicating significant heterogeneity among the pooled studies (Fig. 4). All studies measuring peripheral NK cells used a flow cytometric assay. The studies evaluating uNK cells used immunohistochemistry assay (Parkin et al., 2011) and flow cytometry assay method (McGrath et al., 2009).
NK cells and IVF outcome

Meta-analysis of the two studies that evaluated the role of NK cells in women undergoing IVF treatment showed no significant difference in the live birth rate (RR 0.57; 95% CI 0.06; 5.22; P = 0.62; Fig. 5). The $I^2$ was 64% indicating significant heterogeneity across the studies (Fig. 5). Both were prospective observational studies whereby the target population (women having IVF treatment), with or without raised NK cells or activity, were followed up to the outcomes.

NK cells in women withRM versus controls

Meta-analysis of the four studies that evaluated peripheral NK cell levels expressed as percentages showed a significant difference between...
women with RM versus controls (SMD 1.36; 95% CI 0.04; 2.69; \( P = 0.04 \); Fig. 6a). The \( I^2 \) was 95% indicating significant statistical heterogeneity across the studies. Meta-analysis of the two studies that expressed peripheral NK cells as numbers showed significantly higher levels of peripheral NK cells in women with RM compared with controls (SMD 0.81; 95% CI 0.47; 1.16; \( P < 0.00001 \); Fig. 6b). The \( I^2 \) was 0% indicating no statistical heterogeneity.

Meta-analysis of the six studies that evaluated uNK cells expressed as a percentage of the stromal cells in women with RM versus controls showed no significant difference between the two groups (SMD 0.40; 95% CI 1.24; 2.04; \( P = 0.63 \); Fig. 6c). The \( I^2 \) was 96% indicating significant statistical heterogeneity across the studies. One study that expressed uNK cells as numbers reported significantly higher levels in women with RM compared with controls [mean = 146 per 10 high power field (hpf); SD = 71 versus mean 94 per hpf; SD = 19; \( P = 0.001 \)] (Clifford et al., 1999). Six studies analysed NK cells using flow cytometry (Lachapelle et al., 1996; Michou et al., 2003;...
Figure 6 (a) Peripheral NK cells expressed as percentages in women with RM versus controls. (b) Peripheral NK cells as numbers in women with RM versus controls. (c) uNK cells expressed as a percentage of the stromal cells in women with RM versus controls.

Figure 5 Forrest plots showing the effect of NK cells on IVF outcome—live birth rate.

Shimada et al., 2004; Ntrivalas et al., 2005; Wang et al., 2008; King et al., 2010), five studies using immunohistochemistry (Clifford et al., 1999; Quenby et al., 1999; Michimata et al., 2002; Tuckerman et al., 2007; Parkin et al., 2011) and two studies used the NK cytotoxicity assay to evaluate NK cell activity (Aoki et al., 1995; Shakhar et al., 2003).
Discussion

The results of this review demonstrate no significant difference in the percentage of peripheral or uNK cells in infertile women compared with fertile controls. There was no difference in the percentage of uNK cells in women with RM compared with fertile controls. However, meta-analysis of studies comparing the numbers of peripheral NK cells in infertile women and women with RM versus fertile controls showed significantly higher levels of peripheral NK cell numbers in infertile women and women with RM. There was a significantly higher percentage of peripheral NK cells in women with RM compared with controls. The difference in the results between the NK cells in infertile women or with women with a history of RM and fertile women expressed as numbers or percentage cannot be explained. Only a few studies evaluated NK cells in numbers compared with that expressed as a percentage. There was no significant difference in IVF outcome among women with and without raised NK cells.

Our review represents the first attempt to extensively review the literature and provide a comprehensive estimate of the prevalence of NK cells in women with a history of infertility and RM. The strength of the review lies in the extensive search strategy employed. We performed a funnel plot analysis to assess the publication bias. The funnel plot was symmetrical, indicating that publication and related biases were unlikely. The validity of our results is also directly related to the quality of the primary studies selected through our search. We used the Newcastle–Ottawa Quality Assessment Scale to rate the quality of the included studies (Supplementary data, Table S1). Individual studies scored well on the Quality Assessment Scale.

There were however weaknesses as with any systematic review in that there was clinical heterogeneity between the included studies. Studies varied in the different subpopulations of NK cells measured, in the assay and the immunohistocytochemical methods used to detect NK cells. Other factors likely to influence the results include: the diurnal variation in NK cells (Petitto et al., 1992), parity of women (Gabrilove et al., 1988), whether the blood samples evaluated were fresh or had been frozen (Reichert et al., 1991; Plackett et al., 2004) and the anxiety/stress levels of women (Benschop et al., 1998). The results of the studies also varied in their expression of NK cells as numbers or percentages. The multifactorial aetiologies in the infertile population could also account for the clinical heterogeneity among the studies. The studies in the literature that evaluated both uNK and peripheral NK cells were varied in their methodology. Few studies were observational (Shakhar et al., 2003; van den Heuvel et al., 2007; Tuckerman et al., 2007) and some studies were cohort controlled (Opsahl et al., 1994; Lachapelle et al., 1996; Shimada et al., 2004). The assays used for the analysis of uNK cells also varied (flow cytometric assay, immunohistocytochemistry) in different studies and the best assay method is still uncertain. Flow cytometry measures the binding of NK cells to targets (Garcia-pennarubia et al., 1989; Cavarec et al., 1990; Vitale et al., 1991) and NK cell mediated cytotoxicity (Papa et al., 1988; Vitale et al., 1989). The count of the NK cells will depend upon the setting of the lymphocyte gate on the flow cytometer and will vary in different studies (Rai et al., 2005). On the other hand, immunohistocytochemistry uses monoclonal antibodies to target specific protein antigens in the cell. There is a variation among the published studies with regard to whether NK cells are measured in numbers, percentages or ratios. The reference range of NK cells in infertile and fertile women is also a contentious issue in the literature, with a range as high as 69.69–99.68 in the fertile control group to 79.89–99.48 in the infertile group (Sacks et al., 2012). In some studies, the reference range of NK cells in the different groups has not been mentioned (Lukassen et al., 2003). There is no consensus in the published literature as to what the definition of a raised level of NK cells should be: 10, 12 or 18%.

To address the clinical heterogeneity amongst the studies, we carried out a subgroup analysis of studies of peripheral and uNK cells expressed either as a percentage or whole numbers in the different patient groups. Subgroup analysis of studies evaluating peripheral NK cells expressed as numbers showed significantly higher levels in the infertile and RM groups compared with fertile controls. However, there were only five studies addressing this and further studies are required. The subgroup analysis of studies evaluating peripheral NK cells expressed as percentages showed no difference between the infertile groups and fertile controls. However, one of the studies (Sacks et al., 2012) had a higher number of patients (n = 184) compared with the other five studies included in the subgroup analysis of peripheral NK cells expressed as percentages. This study could skew the results and hence no firm conclusion can be drawn from this analysis. However, more studies with larger sample sizes are required to address this valid clinical question.

Peripheral blood NK cell measurement has been extensively used to guide immunotherapy (Rai et al., 2005). However, it is unclear as to what an abnormal NK cell level is from the published literature for both the infertile and RM populations. A peripheral NK cell level of 12% of all lymphocytes has been regarded as the cut-off between a raised and a normal level in infertile women (Beer et al., 1996), and this figure is well within the normal range (up to 29%) as reported by others (Edukaite et al., 2004). Hence, infertile women with entirely normal results are likely to be labelled as having raised NK cell levels. The prevalence of NK cells in women with RM is unclear. A recent study had concluded that women with RM had a significantly higher NK percentage than controls (King et al., 2010). A receiver operating characteristic curve analysis found that an NK percentage of 18% was highly specific for women with RM (97.0%), and defined 12.5% of women with RM as having an elevated NK cell percentage, compared with 2.9% of controls. On the other hand, studies have also shown that measurement of CD 56 NK cells (expressed as a percentage) in peripheral blood tends to have a higher specificity than sensitivity and therefore is an inaccurate screening tool (Coulam et al., 1995; Yamada et al., 2003).

It has been suggested that CD 56” uNK cells play an important role in implantation and that an increase in cytotoxic peripheral and uNK cells can affect IVF outcome (Fukui et al., 1999). Although uNK cells are likely to be more significant than peripheral NK cells in the process of implantation, we found only two studies evaluating uNK cells in infertile women (McGrath et al., 2009; Parkin et al., 2011) and no studies addressing the role of uNK in women undergoing IVF.

Our review represents the first attempt to review extensively the literature and provide a comprehensive estimate of the prevalence of NK cells in women with a history of infertility and RM. The results of our systematic review are similar to the findings of a recent review by Tang et al. (2011) which addressed the role of NK cells and pregnancy outcomes in women with RM and in women following IVF treatment; this review concluded that abnormal peripheral NK cells or uNK cells did not predict adverse pregnancy outcomes of miscarriage or implantation failure in women with RM or infertility. However, Tang et al. (2011) did mention that the studies included in their review were underpowered and had...
significant heterogeneity between the studies in terms of inclusion criteria and methodology of NK cell analysis and therefore could not address the clinical question adequately.

In conclusion, the immune system is complex and one variable, such as NK cell levels, cannot predict outcome in either women with infertility or RM. NK cell activity is probably only one measure of the overall immune milieu in the infertile women. It is important to understand that NK indices do not reflect specific immune responses to pregnancy and NK cell numbers and activity can fluctuate according to different variables, such as hormonal effect, exercise, time of day and sympathetic response to stressors. Study results may vary depending on laboratory techniques, sample management or selection of study population. This meta-analysis provides an insight into the lack of evidence for the diagnostic workup of using NK cells to enhance female reproductive performance. Drawing clear guidelines for the management of elevated NK cells in infertile women or women with RM is difficult due to the paucity of large randomized trials aimed at determining which patients may benefit from immunotherapy. In conclusion, we do not yet have enough conclusive data to allow us to reach evidence-based conclusions, and greater efforts should be made to understand the value, if any, of these tests as well as the link between test results and use of immune therapy. Further studies are needed to explore the underlying role and mechanisms of action of NK cells in RM, infertility and other reproductive immunopathologies.

Supplementary data
Supplementary data are available at http://humupd.oxfordjournals.org/.

Authors’ roles
S.S. and S.K.S involved in study design, analysis, manuscript drafting and critical discussion; the first author (S.S.) and senior author (S.K.S.) had full access to all the data used for this meta-analysis and took final responsibility for submission for publication.

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Conflict of interest
S.S. and S.K.S. declare that they have no conflict of interest.

References
Baczewski T, Kurzawa R. Immunophenotypic profiles of peripheral blood lymphocytes on the day of embryo transfer in women undergoing in vitro fertilization. Folia Histochem Cytobiol 2007;45(Suppl.1):573–577.
Kwak-Kim JY, Gilman-Sachs A, Kim CE. T helper 1 and 2 immune responses in women with recurrent miscarriage: an insight into the lack of evidence for the diagnostic workup of such as hormonal effect, exercise, time of day and sympathetic response to stressors. Study results may vary depending on laboratory techniques, sample management or selection of study population. This meta-analysis provides an insight into the lack of evidence for the diagnostic workup of using NK cells to enhance female reproductive performance. Drawing clear guidelines for the management of elevated NK cells in infertile women or women with RM is difficult due to the paucity of large randomized trials aimed at determining which patients may benefit from immunotherapy. In conclusion, we do not yet have enough conclusive data to allow us to reach evidence-based conclusions, and greater efforts should be made to understand the value, if any, of these tests as well as the link between test results and use of immune therapy. Further studies are needed to explore the underlying role and mechanisms of action of NK cells in RM, infertility and other reproductive immunopathologies.

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S.S. and S.K.S involved in study design, analysis, manuscript drafting and critical discussion; the first author (S.S.) and senior author (S.K.S.) had full access to all the data used for this meta-analysis and took final responsibility for submission for publication.

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