



Where and when should natural killer cells be tested in women with repeated implantation failure?

Isabel Santillán^{a,*}, Ignacio Lozano^b, Julia Illán^c, Victoria Verdú^b, Santiago Coca^d, Jose Manuel Bajo-Arenas^e, Felipe Martinez^f

^a Centro Médico Palencia, Spain

^b Ginefiv, Spain

^c Unilabs, Spain

^d Hospital Universitario Gomez Ulla, Spain

^e Hospital Universitario Santa Cristina, Spain

^f Universidad de León, Spain

ARTICLE INFO

Article history:

Received 31 August 2014

Received in revised form 8 December 2014

Accepted 31 December 2014

Keywords:

Implantation

Natural killer cells

Immunohistochemistry

Endometrium

CD56

ABSTRACT

The aim of this study was to identify the candidates for natural killer (NK) testing and to define the best methodology. For this purpose a prospective study was performed on 73 women with repeated implantation failure (RIF). RIF was considered to exist in patients not achieving clinical pregnancy after three transfers with at least one good-quality embryo. Idiopathic RIF was considered to exist in patients in whom thrombophilia, hysteroscopy and endometrial culture were normal, and no chromosomal factor was suspected. Thirty-two of the 73 patients were considered to have idiopathic RIF, and 17 fertile women with children were taken as controls. Immunohistochemical staining for endometrial CD56+ and blood CD56+ or CD16+ NK cells measured using flow cytometry were compared during the mid-luteal phase in both patients and controls. Seventeen out of the 32 patients with idiopathic RIF and only one of the controls had >250 CD56 cells per high power field 400× in endometrial biopsy ($p < 0.001$). The percentage of blood NK cells out of the total lymphocyte population was higher in women with idiopathic RIF ($13.4 \pm 1.2\%$; range, 2.63–29.01) than in controls ($8.4 \pm 0.7\%$; range, 5.72–13.28; $p = 0.026$). There was a positive correlation between blood and endometrial CD56 cells ($\rho = 0.707$; $p < 0.001$). No significant differences were found between patients with other types of RIF and controls. This study suggested that testing for NK cells might be useful in women with idiopathic RIF during the mid-luteal phase.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Despite the new technologies, human implantation remains a barrier in assisted reproduction (Edwards, 2007).

Abbreviations: RIF, repeated implantation failure; NK, natural killer; h.p.f., high power fields of 400× magnification.

* Corresponding author at: Centro Médico Palencia, C/ Lagasca 122, 2D, 28006 Madrid, Spain. Tel.: +34 699488126.

E-mail address: isabel.santillan.palencia@gmail.com (I. Santillán).

At the time of implantation, there seems to be a dialog between the embryo and the endometrium (Roberts et al., 2008). In this dialog, the main role is played by the embryo (Dior et al., 2014). Embryos resulting from sperm and egg donation or those chromosomally normal after preimplantation genetic diagnosis (PGD) are usually considered to be of very good quality (Capalbo et al., 2014). However, sometimes, even after transferring very good quality embryos, pregnancy is not achieved.

The first problem faced when treating repeated implantation failure (RIF) is that there is no well-established

definition (Tan et al., 2005; Coughlan et al., 2014). It is also unclear which tests should be included to detect the reasons for these failures.

As aneuploidy is one of the major factors affecting embryo implantation (Rodrigo et al., 2014), parental karyotyping, sperm fluorescence in situ hybridization (FISH), DNA fragmentation (Enciso et al., 2013), and even preimplantational genetic diagnosis could be recommended for women with RIF (Pagidas et al., 2008; Franssen et al., 2011).

Given that anatomical malformations of the uterus may interfere with normal implantation (Simon and Laufer, 2012), and that a chronic endometritis rate of up to 66.3% upon hysteroscopy has been described (Yang et al., 2014), hysteroscopy and endometrial biopsy may be recommended.

Hypothyroidism (Fumarola et al., 2013) has also been associated with reproductive failure and should therefore be included in the investigation. However, some women with repeated implantation failure do not have any of the above-mentioned factors, still do not achieve pregnancy, and would like to identify the reason. Despite there being many publications describing an increased amount of natural killer cells in both blood and endometrium in women with repeated implantation failure and recurrent miscarriage (Tuckerman et al., 2010; Coulam and Goodman, 2000), natural killer (NK) cell testing is not a recommended clinical practice (Tang et al., 2011; Seshadri and Sunkara, 2013), and not all studies agree that NK levels may be associated with implantation problems (Thum et al., 2005). Unless the diagnostic methodology is clear and standardized, it would be difficult to find a solution because of the lack of clarity regarding who should be included in therapeutic trials. A recent meta-analysis reports elevated peripheral NK cells in infertile women, but not a significant increase in uterine NK cells (Seshadri and Sunkara, 2013). A problem of most investigations is the low number of cases and controls. Controls are often infertile patients. In many publications, repeated implantation failure and recurrent miscarriage tend to overlap (Tang et al., 2011), although they have different physiopathology and immunology. T cells, for instance, decrease significantly in the first trimester in the decidua and are unlikely to play an important role in the maintenance of human pregnancy; however, T cells could be important for implantation where their relative numbers are greater (Johnson et al., 1999), and NK cells increase immediately after implantation and severely decrease by gestational day 6 (Takashima et al., 2013).

As there are great differences in cut-off values, the normal range of uterine NK cells for human embryo implantation is controversial.

The most effective methodology for testing NK cells that can be standardized in routine clinical practice is not clear either. It is not clear if NK cell levels should be measured in blood or in endometrium, and very little is known about the correlation between blood and endometrial levels of NK cells (Laird et al., 2011). Flow cytometry can be used to test both blood and uterine NK cells, but to analyze uterine NK cells, they have to be mechanically or enzymatically disintegrated (Manaster et al., 2008). Therefore, this method does not easily allow NK cells to be counted in a defined

tissue volume and is hard to standardize for application in different centers.

In contrast, immunohistochemistry has been successfully used by Tuckerman et al. (2010) and is usually reported to be the percentage of stained cells from the total number of stromal cells. However, it would be interesting to compare NK cell testing as a percentage, total count per field, and relation to the epithelial glands. A previous meta-analysis has demonstrated that the significance of NK cells is different when they are expressed as numbers or as percentages (Seshadri and Sunkara, 2013). However, it is difficult to identify different phenotypes such as NK and natural killer T (NKT) cells. In particular, bright and dim CD56 cells cannot be distinguished by means of immunohistochemistry, and blood NK cells cannot be studied either. It seems that the total numbers of CD56 cells measured using flow cytometry and immunohistochemistry correlate well (Laird et al., 2011). Therefore, we aimed to study uterine NK cells using immunohistochemistry and blood NK cells using flow cytometry focusing on the total amount of CD56 cells rather than different phenotypes, as this is what can be compared in blood and endometrium.

The objective of this study is to define the candidates for this test and to find the best methodology. We also aimed to establish a reasonable cut-off value for normal uterine NK cells and check if blood NK cells correlated with uterine NK cells.

2. Materials and methods

This prospective study on 90 women had the approval of the local ethics committee and required patients to provide informed consent. Data were obtained from 73 women with RIF and 17 controls (Fig. 1). RIF was defined as no clinical pregnancy after three transfers with at least one good-quality embryo (grade A or B). Most transfers took place on the third day. In case the patient's own oocytes were used, only women under 40 years of age were included.

All patients with RIF went through our repeated implantation failure protocol, which includes the following: karyotyping of both the man and the woman; hysteroscopy; endometrial culture and microbiological analysis of endometrial biopsy; sperm FISH for chromosomes 21, 18, 13, X, and Y; and sperm DNA fragmentation.

We diagnosed a subgroup of these women with idiopathic RIF on the basis of these tests being normal. Exclusion criteria for this subgroup were as follows: uncompleted protocol, suspected chromosomal factor, and any immunological disorder, except for autoimmune hypothyroidism if TSH was under 2.5 with medication. We suspected a chromosomal factor in women over 39 years of age, if there were any abnormal embryos after PGD, and if sperm FISH or DNA fragmentation was altered. Women with diagnosed autoimmune disorders, such as rheumatoid arthritis and lupus erythematosus, were excluded. The women excluded from the idiopathic RIF group were also analyzed and considered as cases of other types of RIF.

Fertile women under 35 years of age with no history of infertility and their own children were taken as controls.

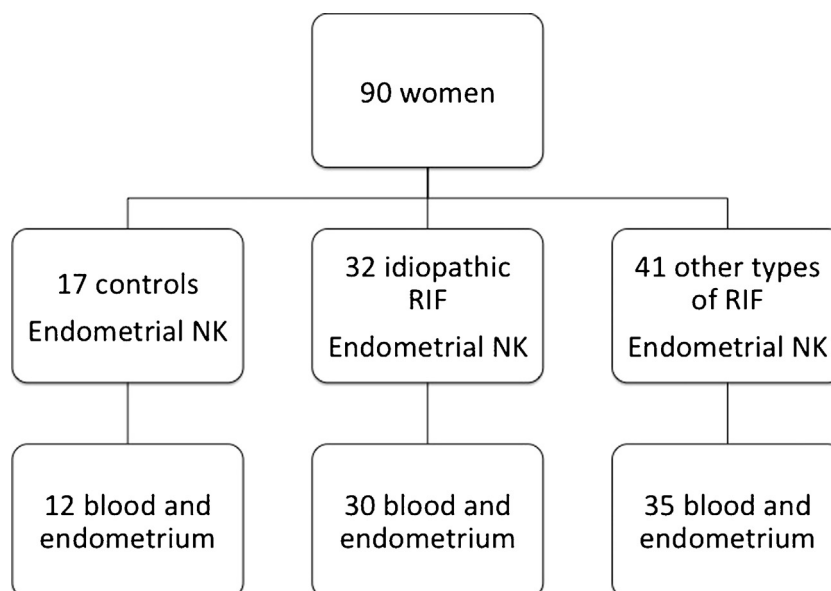


Fig. 1. Flow chart with women with endometrial NK testing and both endometrial and blood NK testing.

Three groups of women were compared: those with idiopathic RIF, those with other types of RIF, and controls.

Endometrial biopsies and blood samples were taken in both RIF patients and controls during the mid-luteal phase, days 19–23 of the natural cycle in women with regular periods, or 5–9 days after a positive ovulation test.

Uterine NK cells in endometrial biopsies were studied using CD56 immunohistochemical staining. The number of CD56-stained cells per 10 high power fields of 400 $\times$ (cell/10 h.p.f.), the percentage of CD56-positive cells out of all stromal cells, and the relation to the epithelial glands were reported. Women were divided into three groups: <150 cells/10 h.p.f., 150–250 cells/10 h.p.f., and >250 cells/10 h.p.f. The relation with the gland was divided as follows: low, moderate, and intense.

Peripheral blood was retrieved at the same time as the biopsy to measure blood NK cells (CD3–/CD56+ or CD16+) using flow cytometry in 77 women (Fig. 1). Flow cytometry was performed in the clinical laboratories Abacid and Unilab. Most samples (67/77) were studied with monoclonal antibodies with four different fluorochromes CD16-FITC, CD56-PE, CD3-PerCP-Cy5,5, and CD45-APC (Becton Dickinson, BD). Total NK cells, NKT, and CD56+ CD16+ were reported. After 15 min of incubation, lysing, centrifuge, and washing, the sample was analyzed using a FACSCanto II (BD) cytometer and the program Infinicyt (Cytognos).

CD56bright were not reported because the aim of measuring blood NK cells was to compare them with endometrial NK cells. As endometrial NK cells were analyzed using immunohistochemistry, CD56 bright cells could not be identified. However, CD56bright cells in blood could also have been reported as shown in Fig. 2. On the other hand, we are looking for a method that can be standardized in routine clinical practice, and in clinical laboratories, technicians are not usually used to reporting

CD56bright NK cells. In Abacid before 2012, blood NK cells were only identified with antibodies for CD3, CD45, and CD56, and in 10 women only CD45, CD56+, and CD3– cells were reported. Cells were given as a percentage of the total number of lymphocytes in all women.

We compared endometrial CD56+ and blood NK cells in the three groups of women: patients with idiopathic RIF, patients with other types of RIF, and controls.

Blood NK cells, blood CD56+ cells, and endometrial CD56+ cells were compared. The sensitivity and specificity of blood NK cells, identifying women with more than 250 endometrial CD56 per h.p.f., were studied. For this purpose we defined normal blood NK cells as <12% of the total lymphocyte population.

Data analysis was carried out using the R statistical environment. Comparisons of the groups were carried out using the chi-squared test (absolute counts) and one-way ANOVA (proportions). Correlations between blood and endometrial NK cells were calculated using Spearman's test. Results are shown as mean $\pm$ standard error of the mean.

3. Results

Thirty-two women were included with idiopathic RIF (mean number of transfers/woman, 5.35), 41 with other types of RIF, and 17 as fertile controls (Fig. 1).

When we compare endometrial CD56 in the three groups, we find that the number of endometrial CD56 cells/10 h.p.f. is significantly different ($p < 0.001$).

This difference is due to a higher number of endometrial CD56+ cells in women with idiopathic RIF than in controls. Seventeen of the 32 women with idiopathic RIF and only one of the 17 controls had >250 CD56-stained cells/10 h.p.f.

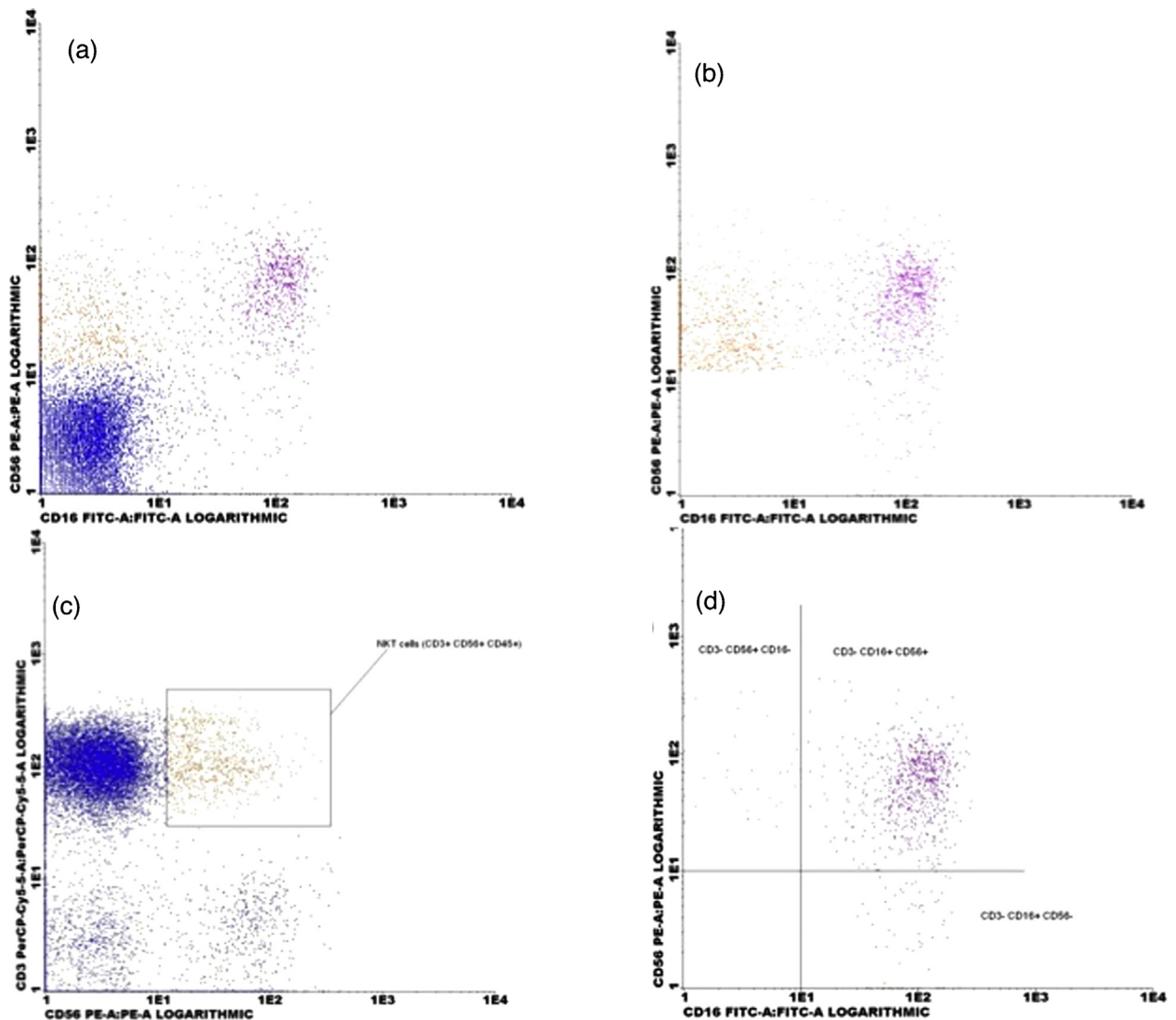


Fig. 2. Blood NK analysis with flow cytometry. Blood NK cells are identified as CD45+, CD3–, CD56+ or CD16+. (a) All lymphocytes (CD45). (b) Both NK and NKT cells. (c) NKT cells (CD3+). (d) Only NK cells (CD3–).

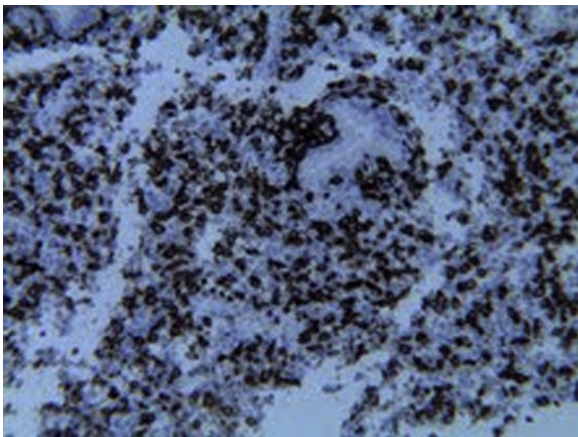


Fig. 3. Massive expression of endometrial CD56 (450 cells in a h.p.f., 400 $\times$) in a woman with RIF.

($p < 0.001$; Fig. 3). No significant difference was observed between other women with RIF (5/41 with >250 CD56+ cells/10 h.p.f.) and controls (1/17; $p = 0.14$).

The percentage of endometrial CD56+ cells out of the total number of stromal cells in women with idiopathic RIF ($19.8 \pm 2.3\%$; range, 5–50) is significantly higher ($p = 0.001$) than that of the controls ($7.4 \pm 1.5\%$; range, 5–30). However, no significant differences were observed between women with other types of RIF ($13.8 \pm 2.2\%$; range, 5–50) and controls ($7.4 \pm 1.5\%$; range, 5–30; $p = 0.128$; Fig. 4a).

The relation between CD56+ cells and the epithelial glands is different in the three groups. In 17 of the 32 women with idiopathic RIF and only two of the 17 controls there was an intense relation with the epithelial glands ($p = 0.007$). Nevertheless, no significant differences were observed between an intense relation with the epithelial glands in women with other types of RIF (8/41) and controls (2/17; $p = 0.238$).

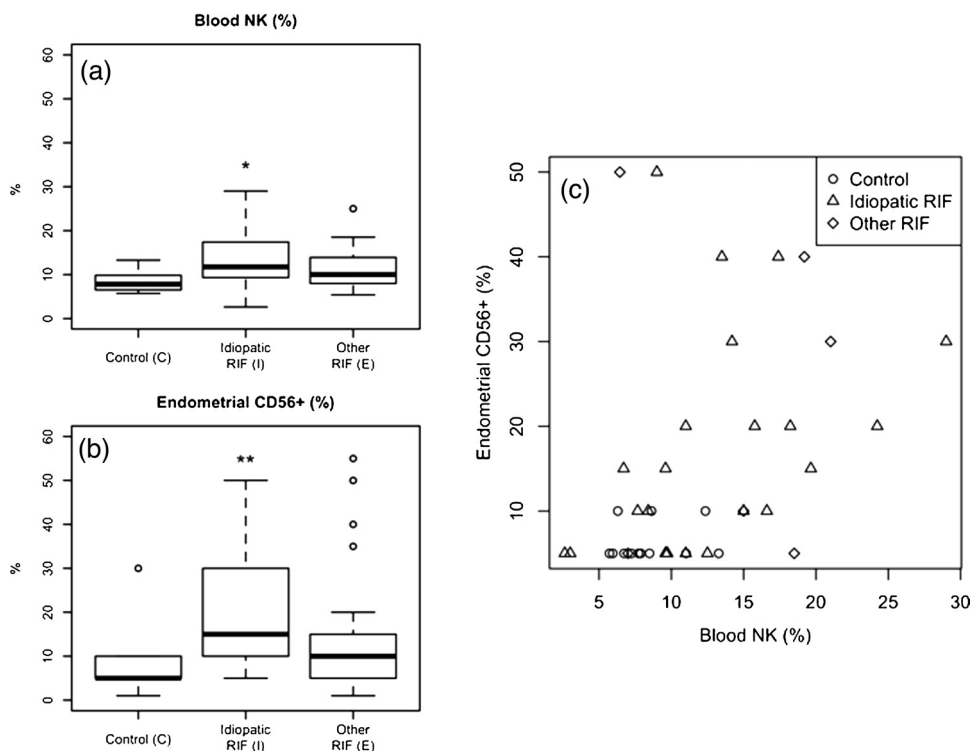


Fig. 4. Box plots of (a) endometrial CD56+ as a percentage of the total stromal cells and (b) blood NK as a percentage of the total lymphocytes in controls, women with idiopathic RIF, and other women with RIF. Asterisks indicate significant differences in idiopathic RIF vs. control group (* $p < 0.05$; ** $p < 0.01$). (c) Scatterplot for the proportions of endometrial CD56 and blood NK cells. The correlation between the two variables was significant, with $\rho = 0.325$ ($p = 0.012$).

Table 1

Predictive value of blood NK measurement to detect a high number of endometrial CD56+ cells.

	<12% blood NK	>12% blood NK	Total
<250CD56/10 h.p.f. 400×	40	14	54
>250CD56/10 h.p.f. 400×	9	14	23
	49	28	77
Sensitivity	14/23	60.87	
Specificity	40/54	74.07	
False positive	14/54	25.93	
False negative	9/23	39.13	

When we studied blood NK cells, we found that the percentage of blood NK cells from total lymphocytes was higher in women with idiopathic RIF ($13.4 \pm 1.2\%$; range, 2.63–29.01) than in controls ($8.4 \pm 0.7\%$; range, 5.72–13.28; $p = 0.026$). Still, no significant differences were observed between women with other types of RIF ($11.1 \pm 0.8\%$; range, 4.64–22) and controls ($8.4 \pm 0.7\%$; range, 5.72–13.28; $p = 0.292$; Fig. 4b).

When we studied NK phenotypes in blood, no differences are found in blood NK like-T cells (NKT) between women with idiopathic RIF ($4.8 \pm 1.2\%$; range, 1.1–41.4) and controls ($3.1 \pm 0.5\%$; range, 1.1–41.4; $p = 0.763$).

When the correlation between blood and endometrial NK cells was studied, we observed that there was a strong positive correlation between total blood NK cells and blood CD56+ and CD16+ cells (Spearman $\rho = 0.919$; $p < 0.001$). The correlation between endometrial CD56 cells and blood NK

cells was positive and weak ($\rho = 0.38$; $p = 0.012$; Fig. 4c), but it increased ($\rho = 0.587$; $p < 0.001$) when considering the idiopathic RIF group alone (Fig. 3). The correlation between endometrial CD56 cells and blood CD56 NK cells was stronger ($\rho = 0.707$; $p < 0.001$).

The sensitivity of blood NK cell testing for identifying women with more than 250 endometrial CD56+ was 14/23 (60.87%) and specificity was 40/54 (74.07%; Table 1).

4. Discussion

Although there are a great number of publications on NK cells and infertility, we think our data contribute to a better understanding of the role of NK cells and repeated implantation after IVF.

Despite 1100 total citations in Seshadri's meta-analysis on NK and fertility, only 22 primary articles were finally included in this systematic review (Seshadri and Sunkara, 2013). In many of these studies RIF and recurrent miscarriage tended to overlap (Thum et al., 2005). Because RIF is not well defined, some considered implantation failure after only one transfer, and infertile patients who achieved pregnancy were taken as controls (Fukui et al., 1999). In our study, idiopathic RIF is well defined and controls are completely fertile women.

Tuckerman et al. also described increased expression of CD56 in 40 women with RIF (Tuckerman et al., 2010), whereas we studied 73 women with RIF. In their study other factors responsible for RIF are not included, and

idiopathic RIF is not defined. Nevertheless, the most important difference in our study is that we also measured blood NK cells in the same women at the same time.

According to Seshadri and Sunkara (2013), no significant differences in the percentage of peripheral or endometrial NK cells are seen in infertile women compared with fertile controls. However, studies comparing the number of peripheral NK cells as $n/\mu\text{l}$ showed significantly higher levels of peripheral NK cell numbers in infertile women and women with recurrent miscarriage. Although we studied women with idiopathic RIF and not those with recurrent miscarriage or just infertile patients, we observed a difference in blood NK cells between patients and controls when expressed as a percentage of total lymphocytes.

There are very few studies comparing blood and endometrial NK cells. Laird et al. (2011) include in their study a comparison of blood and endometrial NK cell levels. In contrast to this publication, we found a positive, albeit weak, correlation between blood CD56+ cells measured with flow cytometry and immunohistochemical staining for CD56+ in the endometrium. The reason for the difference between the findings of Laird et al. (2011) and ours may be that they studied recurrent miscarriage and not idiopathic RIF, and that in their studies, endometrial biopsies in the control group were taken at any time of the cycle. The correlation between blood NK cells and endometrial CD56+ seems to be stronger in women with idiopathic RIF. This could be due to an increased migration of blood NK cells to the endometrium in these women and requires further investigation.

The association between idiopathic RIF and high numbers of NK cells is stronger in the endometrium than in blood although it is also significant in blood. It has been speculated that uterine NK cells come from blood, but recent data suggest that they might also be produced in the uterus from stem cells (Chiossone et al., 2014). This may explain why, despite some correlation, blood NK cell measurement is less sensitive than endometrial NK cell measurement.

Considering the techniques employed in our study, immunohistochemistry has the advantage of allowing visualization of the distribution of the cells within the endometrial stroma, and their relation to the epithelial glands.

The cut-off value for blood natural killer cells is between 12 and 13% of the total lymphocyte population in most studies (Coulam and Goodman, 2000). However, cut-off values for normal uterine NK cells can vary from 5% (Kuroda et al., 2013) to 13.9% (Wong et al., 2014) of the total stromal cells. We suggest a cut-off value of 250 CD56 cells/10 h.p.f., as this is the upper limit of the range, and more than 50% of women with idiopathic RIF compared with just one control had over 250 cells. However, further studies and statistical analysis should be performed before deciding on a clinical cut-off value.

5. Conclusion

Analysis of NK cells may be offered to women with idiopathic RIF. NK cells should be tested in an endometrial biopsy during the mid-luteal phase if possible, although

blood NK testing may also help when endometrial biopsy cannot be performed. However, it may be difficult to include NK testing in routine clinical practice without performing more studies to completely define and diagnose the problem of NK cells, and implantation and cut-off values can be standardized for all laboratories.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Capalbo, A., Rienzi, L., Cimadomo, D., Maggiulli, R., Elliott, T., Wright, G., et al., 2014. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Hum. Reprod.* 29 (6), 1173–1181.
- Chiossone, L., Vacca, P., Orecchia, P., Croxatto, D., Damonte, P., Astigiano, S., et al., 2014. In vivo generation of decidual natural killer cells from resident hematopoietic progenitors. *Haematologica* 99 (3), 448–457.
- Coughlan, C., Ledger, W., Wang, Q., Liu, F., Demirel, A., Gurgan, T., et al., 2014. Recurrent implantation failure: definition and management. *Reprod. Biomed. Online* 28 (1), 14–38.
- Coulam, C.B., Goodman, C., 2000. Increased pregnancy rates after IVF/ET with intravenous immunoglobulin treatment in women with elevated circulating C56+ cells. *Early Pregnancy* 4 (2), 90–98.
- Dior, U.P., Kogan, L., Chill, H.H., Eizenberg, N., Simon, A., Revel, A., 2014. Emerging roles of MicroRNA in the embryo-endometrium cross talk. *Semin. Reprod. Med.* 32 (5), 402–409.
- Edwards, R.G., 2007. Chorionic gonadotrophin, genes and embryonic signals regulating the implantation window. *Reprod. Biomed. Online* 14 (4), 538–540.
- Enciso, M., Alfarawati, S., Wells, D., 2013. Increased numbers of DNA-damaged spermatozoa in samples presenting an elevated rate of numerical chromosome abnormalities. *Hum. Reprod.* 28 (6), 1707–1715.
- Franssen, M.T., Musters, A.M., van der Veen, F., Repping, S., Leschot, N.J., Bossuyt, P.M., et al., 2011. Reproductive outcome after PGD in couples with recurrent miscarriage carrying a structural chromosome abnormality: a systematic review. *Hum. Reprod. Update* 17 (4), 467–475.
- Fukui, A., Fujii, S., Yamaguchi, E., Kimura, H., Sato, S., Saito, Y., 1999. Natural killer cell subpopulations and cytotoxicity for infertile patients undergoing in vitro fertilization. *Am. J. Reprod. Immunol.* 41 (6), 413–422.
- Fumarola, A., Grain, G., Romanzi, D., Del Sordo, M., Bianchini, M., Aragona, A., 2013. Thyroid function in infertile patients undergoing assisted reproduction. *Am. J. Reprod. Immunol.* 70 (4), 336–341.
- Johnson, P.M., Christmas, S.E., Vince, G.S., 1999. Immunological aspects of implantation and implantation failure. *Hum. Reprod.* 14, 26–36.
- Kuroda, K., Venkatakrishnan, R., James, S., Sucurovic, S., Mulac-Jericevic, B., Lucas, E.S., 2013. Elevated periimplantation uterine natural killer cell density in human endometrium is associated with impaired corticosteroid signaling in decidualizing stromal cells. *J. Clin. Endocrinol. Metab.* 98 (11), 4429–4437.
- Laird, S.M., Mariee, N., Wei, L., Li, T.C., 2011. Measurements of CD56 cells in peripheral blood and endometrium by flow cytometry and immunohistochemical staining in situ. *Hum. Reprod.* 26 (6), 1331–1337.
- Manaster, I., Mizrahi, S., Goldman-Wohl, D., Stem-Ginossar, N., Laukry, D., et al., 2008. Endometrial NK cells are special immature cells that await pregnancy. *J. Immunol.* 181 (3), 1869–1876.
- Pagidas, K., Ying, Y., Keefe, D., 2008. Predictive value of preimplantation genetic diagnosis for aneuploidy screening in repeated IVF-ET cycles among women with recurrent implantation failure? *J. Assist. Reprod. Genet.* 25 (2–3), 103–106.
- Roberts, R.M., Chen, Y., Ezashi, T., Walker, A.M., 2008. Interferons and the maternal-conceptus dialog in mammals. *Semin. Cell Dev. Biol.* 19 (2), 170–177.
- Rodrigo, L., Mateu, E., Mercader, A., Cobo, A.C., Peinado, V., Milán, M., et al., 2014. New tools for embryo selection: comprehensive chromosome screening by array comparative genomic hybridization. *Biomed. Res. Int.* 2014, 517125.
- Seshadri, S., Sunkara, S.K., 2013. Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis. *Hum. Reprod. Update* 20 (3), 429–438.

- Simon, A., Laufer, N., 2012. Assessment and treatment of repeated implantation failure (RIF). *J. Assist. Reprod. Genet.* 29 (11), 1227–1239.
- Takashima, A., Ishikawa, F., Kuwabara, T., Tanaka, Y., Kinoshita, T., Ito, M., Kakiuchi, T., 2013. Uterine natural killer cells severely decrease in number at gestation day 6 in mice. *Biol. Reprod.* 89 (4), 101.
- Tan, B.K., Vandekerckhove, P., Kennedy, R., Keay, S.D., 2005. Investigation and current management of recurrent IVF treatment failure in the UK. *BJOG* 112 (6), 773–780.
- Tang, A.W., Alfirevic, Z., Quenby, S., 2011. Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. *Hum. Reprod.* 26 (8), 1971–1980.
- Thum, M.Y., Bhaskaran, S., Bansai, A.S., Shehata, H., Ford, B., Sumar, N., et al., 2005. Simple enumerations of peripheral blood natural killer (CD56+ NK) cells, B cells and T cells have no predictive value in IVF treatment outcome. *Hum. Reprod.* 20 (5), 1272–1276.
- Tuckerman, E., Mariee, N., Prakash, A., Li, T.C., Laird, S., 2010. Uterine natural killer cells in peri-implantation endometrium from women with repeated implantation failure after IVF? *J. Reprod. Immunol.* 87 (1–2), 60–66.
- Wong, A.W., Archer, B., Mariee, N., Li, T.C., Laird, S.M., 2014. Do uterine natural killer cell number in peri-implantation endometrium predict hypertensive disorder in pregnancy in women with a history of reproductive failure? *J. Reprod. Immunol.* 106, 34–40.
- Yang, R., Du, X., Wang, Y., Song, X., Yang, Y., Qiao, J., 2014. The hysteroscopy and histological diagnosis and treatment value of chronic endometritis in recurrent implantation failure. *Arch. Gynecol. Obstet.* 289 (6), 1363–1369.