Hormonal stimulation for IVF treatment positively affects the CD56\textsuperscript{bright}/CD56\textsuperscript{dim} NK cell ratio of the endometrium during the window of implantation

H.G.M.Lukassen\textsuperscript{1}, I.Joosten\textsuperscript{2}, B.van Cranenbroek\textsuperscript{2}, M.J.C.van Lierop\textsuperscript{3}, J.Bulten\textsuperscript{4}, D.D.M.Braat\textsuperscript{1} and A.van der Meer\textsuperscript{5}

\textsuperscript{1}Department of Obstetrics and Gynaecology of the University Medical Center Nijmegen, \textsuperscript{2}Department of Blood Transfusion and Transplantation Immunology of the University Medical Center Nijmegen, \textsuperscript{3}Department of Pharmacology, NV Organon, Oss and \textsuperscript{4}Department of Pathology of the University Medical Center Nijmegen, The Netherlands

\textsuperscript{5}To whom correspondence should be addressed at: Department of Blood transfusion and Transplantation Immunology (603), PO Box 9101, 6500 HB Nijeger, The Netherlands. E-mail: a.vandermeer@abti.umcn.nl

The effects of hormone stimulation for IVF treatment on endometrial receptivity remain controversial. Since CD56\textsuperscript{bright} natural killer (NK) cells in the endometrium positively contribute to implantation and decidualization whereas CD56\textsuperscript{dim} NK cells are negatively associated with reproduction, shifts in the balance between those cells will affect receptivity. Therefore, we compared the leukocyte composition in the endometrium of IVF women (n = 20) with non-pregnant women (n = 18) in a natural cycle, as a parameter for endometrial quality. Biopsies were obtained 7 days after ovulation. Histological dating of the endometrium showed no increased endometrial advancement after IVF treatment as compared to the control group. Flow cytometric analysis of leukocyte subsets showed that hormonal stimulation positively affected the CD56\textsuperscript{bright}/CD56\textsuperscript{dim} ratio in the endometrium by a relative decrease in the cytotoxic CD56\textsuperscript{dim}CD16\textsuperscript{+} NK cell numbers. The relative number of T-cells remained unaffected, while the number of non-T and non-NK cells (i.e. B-cells and macrophages) was higher in the IVF group. These effects were restricted to the endometrium and not observed in peripheral blood. Within the CD56\textsuperscript{bright} population we could identify a distinct subset of NK cells (CD56\textsuperscript{superbright}) that was unique for the endometrium. We conclude that hormonal stimulation for IVF treatment positively affects the CD56\textsuperscript{bright}/CD56\textsuperscript{dim} ratio of the endometrium during the window of implantation and does not affect endometrial advancement.

Key words: CD56/endometrium/IVF/NK cells

Introduction

Ovarian stimulation for IVF treatment results in supraphysiological concentrations of sex steroids during the follicular and luteal phase of the menstrual cycle. The effect on endometrial receptivity remains controversial. Both positive and negative effects on pregnancy rates after IVF have been reported (Chenette et al., 1990; Paulson et al., 1990; Simon et al., 1995; Macklon and Fauser, 2000).

To date there have been limited means to define endometrium quality. One parameter is the histological maturation of the endometrium in relation to the menstrual date. It has been stated that extreme endometrial advancement (>2 days) results in lower pregnancy rates (Klentzeris et al., 1992; Ubaldi et al., 1997; Kolibianakis et al., 2002). Of interest with regard to IVF is that high levels of progesterone were found to be associated with advanced endometrial histology (Garcia et al., 1984; Chekowskii et al., 1997; Santoro et al., 2000), dated by criteria of Noyes et al. (1950).

Based on recent insights into natural killer (NK) cell function, the leukocyte composition in the endometrium could well be considered an alternative parameter for endometrial quality. During the menstrual cycle, uterine NK cells increase markedly in number after ovulation and reach a peak in the late secretory phase. In pregnancy their numbers remain high during early gestation, ~70% of the stromal leukocytes, and they come into close contact with invading extravillous trophoblast cells (Klentzeris et al., 1992; Loke and King, 1997; Santoro et al., 2000). The localization and the large numbers of NK cells found in the decidua during pregnancy suggest an important role in the decidualization (King, 2000).

The uterine NK cells (uNK) that are reputed to play an important role in implantation have a phenotype that differs from most peripheral blood NK cells. These so-called CD56\textsuperscript{bright} NK cells express the NK cell marker CD56 at high levels and lack the marker CD16. These NK cells have low cytotoxic activity but are potent producers of a variety of immunoregulatory cytokines and angiogenic growth factors contributing to endometrial angiogenesis (Cooper et al., 2001; Jacobs et al., 2001; Li et al., 2001). In contrast, most peripheral blood NK cells, but also a small fraction of the uterine NK cells, is of the CD56\textsuperscript{dim}CD16\textsuperscript{+} phenotype with high cytolytic activity and low cytokine production (Jacobs et al., 2001). This cytotoxic CD56\textsuperscript{dim} NK cell population is negatively associated with pregnancy outcome (Beer et al., 1996; Fukui et al., 1999; Emmer et al., 2000; Ntrivalas et al., 2001).

Activation of NK cells is tightly regulated by a set of activating and inhibitory receptors on the cell surface. These receptors interact with HLA molecules expressed on the invading trophoblast (such as HLA-C, HLA-E and HLA-G) and thereby affect cytokine production and cytolytic activity of maternal uNK cells (Hunt et al., 1997).
2000; King et al., 2000; Kanai et al., 2001; Rieger et al., 2002). It is believed that each individual NK cell and the NK cell subsets expresses a unique combination of natural killer cell receptors depending on their functional role.

During the menstrual cycle as well as throughout pregnancy the leukocyte numbers vary, therefore an interaction with sex steroids is suggested, although it is not clear yet whether this is a direct or an indirect mechanism. DeLoia et al. (2002) have shown that the total number of lymphocytes in endometrium as well as in peripheral blood, particularly the NK cells, increases under the influence of supraphysiological estrogen levels.

To determine the influence of ovarian stimulation on shifts in endometrial leukocyte populations, and in particular the NK cell subsets, we compared leukocyte populations in the endometrium of IVF patients with naturally cycling women during the window of implantation. By analysing shifts in the beneficial CD56+ NK cells and the harmful CD56dim NK cells, we want to answer the question whether hormonal stimulation during IVF treatment improves or deteriorates the quality of the endometrium.

**Materials and methods**

**Patients**

In the study group (IVF group), endometrial tissue was obtained from 20 women participating in an IVF/ICSI programme. These 20 women had no embryo transfer. Embryo transfer was omitted due to total fertilization failure (nine with male factor infertility, seven with idiopathic infertility, three with tubal factor infertility, one with polycystic ovary syndrome). In one case, no embryos were transferred due to severe ovarian hyperstimulation syndrome. In 16 patients, IVF was carried out and four patients were treated by ICSI.

In the control group, endometrial biopsies were obtained in a natural cycle from 18 non-pregnant women. These women had a regular cycle. They had a partner with severe male infertility and were recruited from the waiting list for ICSI. One woman was a healthy volunteer with no history of subfertility.

Characteristics of both groups are summarized in Table I.

All women gave informed consent according to the Medical Ethical Review Committee of the University Medical Center Nijmegen.

**IVF procedure**

Pituitary down-regulation (long protocol) was achieved using a GnRH analogue (Decapeptyl®; Ferring, The Netherlands). Multiple follicular stimulation was realized by recombinant FSH (Puregon®; Organon, The Netherlands). Thirty-six hours after hCG injection (Pregnyl®; Organon) we performed transvaginal oocyte retrieval under systemic analgesia (10 mg oxazepam orally and 1 mg alfentanil, i.v.). The retrieved oocytes were inseminated or ICSI was performed according to the method described by Van Steirteghem et al. (1995). The morning following injection or insemination, fertilization of the oocytes was judged. The luteal phase was supported by three doses of progesterone (200 mg; Progestan®; Organon, The Netherlands) intravaginally daily from the day of oocyte retrieval until the day of the endometrial biopsy.

**Endometrial biopsy**

In the IVF group, endometrial tissue was obtained by microcurettage using a Pipelle de Comier (Prodimed, France) 6 days after oocyte retrieval. In the control group, an endometrial biopsy was taken 7 days after the endogenous LH surge was detected, tested daily, with a urinary semiquantitative monoclonal antibody-based kit (Clearplan; Unipath Ltd, UK). Median weight of the biopsies was not significantly different between the IVF group (1.1 g) and the control group (0.7 g).

Of the first three IVF patients and two controls, all the collected material was used immediately for isolation of uterine mononuclear cells. In the following 17 biopsies in the IVF group and 16 biopsies in the control group, a part of the tissue was fixed for formaldehyde and used for histological examination. Endometrial histology was analysed by Noyes’ (1950) criteria by a single observer who was blinded to the treatment group. In the IVF group, the day of oocyte retrieval was considered the day of ovulation (day 0), while in the control group day 0 was defined 24 h after the LH surge. The endometrium was considered out of phase if there was a discrepancy of ≥3 days between the observed and expected endometrial maturation, as this was the published margin of error described by Noyes’ (1950) criteria. Glandular–stromal dissociation was observed in one IVF patient. Peripheral blood samples were drawn immediately before the endometrial biopsy was taken to assess lymphocyte populations and determination of estradiol and progesterone levels.

**Isolation of uterine mononuclear cells**

Endometrial tissue was suspended in a very small volume of Roswell Park Memorial Institute 1640 medium with glutamax supplemented with pyruvate containing 100 IU/ml penicillin, 100 mg/ml streptomycin (all from Gibco, UK) and 10% heat-inactivated pooled human serum and then disrupted very carefully by mincing between two scalpels. This was performed very thoroughly until the suspension only contained fragments of ≤0.5 mm. The cell suspension was then filtered through a 70 μm sieve while gently pushing with the back of a plunger. Living mononuclear cells were isolated from the cell suspension by density centrifugation (Lymphoprep; Nycomed, Norway) yielding a median of 1.0 × 10⁶ mononuclear cells per biopsy in the IVF group and 0.5 × 10⁶ in the control group (P = non-significant). Median weight of the biopsies was not significantly different between the IVF group (1.1 g) and the control group (0.7 g). Peripheral blood mononuclear cells (PBMC) were isolated from blood samples by density centrifugation (Lymphoprep).

**Monoclonal antibodies and flow cytometry**

Cells were phenotypically analysed by a direct one-step triple labelling procedure. The following monoclonal antibodies were used: CD3 (clone UCHT1) conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE), CD16 (clone D130C) conjugated with FITC, CD45 (clone T29/33) conjugated with PE, all from Dako (Denmark), CD56 (clone NKH1) conjugated with PE-cytochrome 5 (Cy5), CD158a (clone EB6) conjugated with PE, 158b (clone GL183) conjugated with PE, NKG2A/CD94 (clone Z199) conjugated with PE, all from Dako (Denmark), CD56 (clone NKH1) conjugated with PE-cytochrome 5 (Cy5), CD158a (clone EB6) conjugated with PE, 158b (clone GL183) conjugated with PE, NKG2A/CD94 (clone Z199) conjugated with PE, all from Coulter Immunotech (Franz). The ILT-2 antibody was a kind gift from Dr López-Botet (Spain) and was stained indirectly using an GAM-PE antibody. The samples were run on a Coulter Epics XL Flowcytometer (Beckman Coulter, USA), and 10 000 events were collected based on live leukocyte gating as indicated by propidium iodide (PI, 5 mg/ml) in a forward and side-scatter dot plot. Backgating on CD3 and CD56 confirmed that practically all lymphocytes were located in the gate. Isotype-matched antibodies were used to define marker settings. Analysis of the data was performed using Coulter Epics Expo 32 software (Beckman Coulter). CD45 was used as common leukocyte marker and T-cells were identified as CD3+CD56- cells, NK cells as CD56+CD3- cells and NKT cells as CD3+CD56+ cells.

**Immunohistochemistry**

Formalin-fixed and paraffin-embedded endometrial biopsies were used. From 17 IVF patients and 16 controls a 4 μm thick paraffin section was stained using the monoclonal antibody CD45RB, clone PD7/26 (Dako, Denmark). The tissue sections were stained with the recently developed and highly sensitive, specific and ready-to-use PowerVision kit (ImmunoLogic, The Netherlands) in a dilution 1:800 in phosphate-buffered saline. CD45+...
leukocytes were scored by counting five randomly selected microscope fields at a magnification of ×400. The average number of cells per ×400 field is reported. The slides were scored by three investigators blinded to the identity of the samples.

Statistical analysis

NK, NKT, T-cell, CD45⁺CD3⁻CD56⁻ cell numbers and the NK subsets were expressed as a percentage of the number of CD45⁺ cells. The Mann–Whitney U-test was used to test for statistical differences in mean percentages of leukocyte populations and FSH value between the IVF and the control group. The t-test for independent samples was used, after log-transformation if appropriate, to test for statistically significant differences of mean values of age, estradiol and progesterone levels between the IVF and control group. All tests were two-tailed with a confidence interval of 95% (P < 0.05). All tests were performed using the Statistical Package for Social Sciences.

Results

IVF treatment does not result in endometrial advancement

As expected, the median levels of estradiol and progesterone in peripheral blood were significantly higher in the IVF group as compared to the control group (3700 pmol/l and 170 nmol/l versus 560 pmol/l and 37 nmol/l respectively (P < 0.0001). There was no difference in age, obstetrical history and basal FSH level between the IVF and control groups (Table I).

Endometrial histology can be used as a measure of endometrial quality since extreme endometrial advancement (≥ 3 days) results in lower pregnancy rates. To determine whether hormone treatment affected endometrial advancement, biopsies were scored according to the criteria of Noyes et al. (1950). There was no significant difference in number of patients with endometrial advancement between the IVF group and the control group (37 and 19% respectively, Figure 1). Thus, hormone treatment did not affect endometrial advancement.

Hormone treatment shifts the balance away from the cytotoxic CD56dimCD16⁻ population

Another way to define endometrial quality could be by defining the leukocyte composition of the endometrium and more particular NK cell ratios. During implantation, CD56bright NK cells are reputed to play an important role whereas CD56dim NK cells are mostly negatively associated with reproduction. Therefore, we determined absolute numbers of leukocytes in endometrial biopsies, and, more importantly, we assessed leukocyte subpopulations by flow cytometric analysis as a measure of the total population of leukocytes (i.e. CD45⁺).

CD45 staining of paraffin sections of endometrial biopsies showed that hormone treatment resulted in higher leukocyte levels (Figure 2). The average number of leukocytes in the IVF group was 5.7 per ×400 field compared to 2.7 in the control group (P < 0.05).

We found that the proportion of the uterine CD56dimCD16⁺ NK cells was significantly lower in IVF patients as compared to the controls (6.9 and 13.7% respectively, P < 0.01, Table II). The proportion of CD56bright NK cells (CD56⁺/CD16⁻) was not different between IVF patients and controls in the uterus (28.8 and 33.5% respectively, Figure 3). These data indicate a shift in the ratio towards the beneficial CD56bright NK cells and away from the harmful CD56dim NK cells.

The relative number of uterine T-cells remained unaffected by ovarian stimulation (31.7 and 36.1% respectively). The level of CD45⁺CD3⁻CD56⁻ cells, i.e. mainly monocytes and few B-cells, was significantly higher in the endometrium of IVF patients as compared to the control group (27.6 and 11.6%, P < 0.001, Table II).

Similar results were obtained when the analyses were limited to patients that were shown to be in the mid-secretory phase, seven in the IVF group and 10 in the control group (Figure 1). The percentage CD56bright NK cells was significantly lower in the IVF group as compared to the control group (6.5 and 15.1%, P < 0.01.

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**Figure 1.** Endometrial maturation. The data are illustrated as a frequency distribution of the endometrial glandular dating expressed as observed (Noyes’ criteria) minus expected (calculated from day of ovulation) endometrial maturation in days per patient in the IVF group and the control group.

**Figure 2.** The endometrium of hormone-treated women contains a higher number of leukocytes. The absolute number of CD45⁺ cells per ×400 field was scored per patient by counting five randomly selected microscope fields at a magnification of ×400. The average number of cells per ×400 field is reported.
The proportion of cytotoxic CD56^dim NK cells is lower in the endometrium of hormone-treated women. NK cell populations in endometrium in all patients (upper panel) and in patients in the mid-secretory phase (lower panel) derived from hormone-treated women (IVF patients) compared to control women. Cells were analysed by flow cytometry, gating on the living lymphocytes. Data are expressed as percentage of the whole leukocyte population (i.e. CD45^+). CD56^dim = CD16^-/CD56^+; CD56^bright = CD16^-/CD56^++. *P < 0.05. All tests were performed by the Mann–Whitney U-test. **P < 0.01.
NK cell population. One subset had a similar expression of CD56 as peripheral blood CD56^bright NK cells and the second one had a clearly higher expression of the marker CD56 (Figure 5). Based upon this finding, we defined three distinct NK cell populations in the uterus, i.e. CD56^dim, CD56^bright and CD56^superbright. The CD56^superbright/CD16^+ was unique for the uterus and was not found in peripheral blood (Figure 5).

To further characterize whether these uterine CD56^superbright/CD16^+ NK cells differed from uterine CD56^bright NK cells, we determined the expression of various NK cell receptors that are involved in interactions with HLA molecules on trophoblast cells, namely CD158a and CD158b that bind to HLA-Cw, ILT-2 that binds to HLA-G and NKG2a that binds to HLA-E. The data show that the CD56^superbright^ cells do indeed differ from the CD56^bright^ NK cells with respect to NK cell receptor expression (Figure 6). A significantly higher number of CD56^superbright^ NK cells express CD158a, CD158b, ILT2 and NKG2A as compared to CD56^bright^ NK cells (Figure 7, P < 0.05). Also, the level of expression of NKG2A was significantly higher in the CD56^superbright^ NK cell population as compared to CD56^dim^ and CD56^bright^ NK cells. There was a trend towards a higher level of CD158a, CD158b and ILT2 expression in the CD56^superbright^ NK cells as compared to the CD56^bright^ NK cells (Figure 7).

In conclusion, many CD56^superbright^ NK cells express NK cell receptors and practically all cells of this subset are NKG2A positive. These cells were only found in the endometrium and not in peripheral blood. In contrast, only few uterine CD56^bright^ NK cells express the NK cell receptors CD158a, -b and ILT2, although the majority of cells (80%) are NKG2A positive. This pattern is comparable to peripheral blood CD56^bright^ NK cells.

There was no significant difference in CD56^bright^ NK cell number between the IVF and control groups (14.8 and 19.7%, respectively). This also applies for the number of CD56^superbright^ NK cells between the IVF and control groups (13.8 and 13.8%, Figure 8).

**Discussion**

A critical event at the onset of a pregnancy is the implantation of the embryo in the uterus. At that time there is believed to be an active dialogue between fetal trophoblast cells and uterine lymphocytes that are present in the endometrium. During IVF treatment, a patient is exposed to supraphysiological levels of sex steroid hormones either directly by administration (progesterone) or indirectly due to multiple follicle growth (estradiol and progesterone). In the current study, we determined whether treatment with exogenous hormones affected the composition of the lymphocyte populations in the endometrium during the window of implantation. We paid special attention to the uterine NK cell subset, since it is thought that NK cells play an important role in human reproduction (Moffett-King, 2002). Our data show that ovarian stimulation leads to a shift in the ratio of CD56^dim^/CD56^bright^/CD16^+ NK cells, i.e. a relative decrease in the cytotoxic CD56^dim^/CD16^+ NK cell population.

From the literature it is known that the CD56^dim^/CD16^+ NK cell subset is negatively associated with reproductive events (Beer et al., 1996; Kwak et al., 1996; Fukui et al., 1999; Emmer et al., 2000; Ntrivalas et al., 2001). Fukui et al. (1999) reported higher cytotoxicity and CD56^dim^/CD16^+ NK cell count in endometrial tissue of IVF patients experiencing implantation failure or an abortion compared to an ongoing pregnancy. In contrast, the CD56^bright^/CD16^+ NK cell population is associated with successful implantation and placentation maturation (King, 2000) and is supposed to be crucial at the time of implantation by controlling trophoblast invasion and the production of immunoregulatory cytokines (King et al., 1998; Rieger et al., 2002). An important function of the invading trophoblast cells is vascular transformation of the spiral arteries to provide an adequate blood supply to the fetus and placenta. Our data suggest that hormone treatment shifts the balance towards the CD56^bright^ cells and away from the cytotoxic CD56^dim^ NK population and would thus positively affect endometrial quality.

The shift in NK cell subpopulations does not appear to be due to differences in endometrial maturation between hormone-treated women and control women. First, we found no significant difference in histological dating between those two groups. Second, when the analyses were limited to women who were in the mid-secretory phase, similar results were obtained, i.e. a decrease in the proportion of CD56^dim^ NK cells.

We discovered even a third subset of NK cells in the endometrium at the time that it is receptive for implantation, which has not been described previously. We showed that, based upon the NK cell marker CD56 and NK cell receptor expression, the CD56^bright^ population could be subdivided into two different populations. The so-called CD56^superbright^ cells, which are CD16^+^, were detected only in the endometrium and not in peripheral blood. Furthermore, in contrast to both uterine and peripheral CD56^bright^ NK cells, a large proportion of CD56^superbright^ NK cells express NK cell receptors for HLA-C and G molecules that are present on the invading trophoblasts. Practically all CD56^superbright^ express NKG2A at high levels. This latter receptor binds HLA-E, a non-classical class Ib molecule. These features of CD56^superbright^ NK cells suggest different functional properties as compared with CD56^bright^ cells. Preliminary data that we obtained from a single specimen of decidua of a 13 week pregnant woman with a cervical carcinoma indicate that the CD56^superbright^ NK cell population can also be found in the decidua. Perhaps it is this CD56^superbright^ subset of the CD56^bright^ NK cells that is responsible for the favourable effects on reproduction as described above.

It has been shown by us as well as others that the absolute number of leukocytes in the endometrium increases under the influence of sex steroids (DeLoia et al., 2002). This would suggest that
the shift in the CD56^{dim}/CD56^{bright} ratio is caused by specific recruitment of the less cytolytic NK cell population. So far the mechanisms behind this specific recruitment of leukocyte subsets remains uncertain. Estrogen receptors have been detected on NK cells (Henderson et al., 2003), although there is some controversy about this (Stewart et al., 1998). Alternatively, chemokine receptor expression on CD16^- cells (Hanna et al., 2003) and on CD56^{bright} cells and monocytes (Drake et al., 2001) might be involved in specific homing to invading extravillous trophoblast cells. Differential expression of chemokine receptors by human CD56^{bright} and CD56^{dim} NK cells indeed suggests that these subsets may home to different microenvironments in vivo (Robertson, 2002).

A feature of endometrial quality is the receptivity determined by histological dating of endometrial tissue by criteria of Noyes et al. (1950). Biopsies, in natural as well as in ovarian-stimulated cycles, which are out of phase by \( \geq 3 \) days, are associated with a reduction

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**Figure 6.** CD56^{superbright} NK cells differ from CD56^{bright} NK cells with respect to number and expression levels of NK cell receptors. Uterine and peripheral blood mononuclear cells were stained with CD56^PC5 and CD3^FITC in combination with CD158a, CD158b, ILT2 or NKG2A labelled with phycoerythrin. Double staining of CD56 and the different NK cell receptor is shown for a single representative example. CD56^+, CD56^{++} and CD56^{+++} represent CD56^{dim} NK cells, CD56^{bright} NK cells and CD56^{superbright} respectively.
in clinical pregnancy rates (Davies et al., 1990; Klentzeris et al., 1992; Kolibianakis et al., 2002). In contrast to others (Garcia et al., 1984; Ben Nun et al., 1992; Chetkowski et al., 1997), we did not find significantly more cases of endometrial advancement in the IVF group as compared with the control group, although we are aware of the small numbers of biopsies investigated. So, we suppose that in our study the supraphysiological sex steroid concentrations have no deleterious impact upon implantation.

In summary, we conclude that hormonal stimulation for IVF treatment positively affects the CD56\textsuperscript{bright}/CD56\textsuperscript{dim} ratio of the endometrium during the window of implantation by a relative decrease in the cytotoxic CD56\textsuperscript{dim}/CD16\textsuperscript{+} NK cell number. Further studies are needed to investigate the relationship between NK cell subset composition of endometrium and pregnancy rates after ovarian stimulation in order to improve stimulation protocols in assisted reproductive technology.

Figure 7. CD56\textsuperscript{superbright} NK cells differ from CD56\textsuperscript{bright} NK cells with respect to number and expression levels of NK cell receptors. Upper panel (four graphs) represents the percentage of NK cells expressing a particular receptor expressed as percentage of the total population of that particular NK cell (CD56\textsuperscript{dim}, CD56\textsuperscript{bright} or CD56\textsuperscript{superbright}). Lower panel (four graphs) represent expression level per NK receptor positive cell (expressed as mean fluorescence intensity). The line represents the mean value. CD56\textsuperscript{dim} is defined as CD16\textsuperscript{+}/CD56\textsuperscript{+}, CD56\textsuperscript{bright} is CD16\textsuperscript{−}/CD56\textsuperscript{++} and CD56\textsuperscript{superbright} is CD16\textsuperscript{−}/CD56\textsuperscript{++}. Gating for the CD56\textsuperscript{superbright} population was based upon maximal Cy5 signal found in the peripheral blood NK cell population.
CD56supbright NK cell levels do not differ between hormone-treated women and controls. Cells were analysed by flow cytometry, gating on the living lymphocytes. Data are expressed as percentage of the whole leukocyte population (i.e. CD45+). CD56supbright = CD16+/CD56++ and CD56supbright = CD16+/CD56++++. Gating for the CD56supbright population was based upon maximal Cy5 signal found in the peripheral blood NK cell population.

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