# FINAL REPORT

(01. August 2009. – 31. March 2011)

„The mechanisms of immunological tolerance during pregnancy: impaired maternal tolerance during pre-eclampsia and habitual abortion"

OTKA-NFF 78790
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## 1. PUBLICATIONS WITH "NNF-OTKA SUPPORT" IN THE ACKNOWLEDGEMENT

**1.1.** Miko E, Manfai Z, Meggyes M, Barakonyi A, Wilhelm F, Varnagy A, Bodis J, Illes Z, Szekeres-Bartho J and **Szereday L**. The possible role of NK and NKT-like cells in implantation failure after in vitro fertilization. Reprod Biomed Online 2010; 21: 750-756.
**Impact factor: 2,38**

**1.2.** Brubel R, Boronkai A, Reglodi D, Racz B, Nemeth J, Kiss P, Lubics A, Toth G, Horvath G, Varga T, Szogyi D, Fonagy E, Farkas J, Barakonyi A, Bellyei Sz, **Szereday L**, Koppan M, Tamas A. Changes int he expression of Pituitary Adenylate Cyclase Acivating Polypeptide (PACAP) int he human placenta during pregnancy and its effects on survival of JAR choriocarcinoma cells. J. Mol. Neurosci. 2010; 42: 450-458.
**Impact factor: 2,72**

**1.3.** Barakonyi A, Weisdorn R, Miko E, Varga P, Bodis J, Szekeres-Bartho J, **Szereday L**. Expression profiles of peripheral CD160+ lymphocytes during the course of healthy human pregnancy. Am. J. Reprod. Immunol 2011 Jan 31. doi: 10.1111/j.1600-0897.2011.00983.x. [Epub ahead of print]
**Impact factor: 2,172**

**1.4.** Banati M, Hosszu Zs, Trauninger A, **Szereday L**, Illes Zs. Enzyme replacement therapy induces T cell responses in late-onset Pompe disease. Muscle & Nerve (in press) 2011.
**Impact factor: 2,287**

**1.5. Szereday L**, Miko E, Meggyes M, Barakonyi A, Farkas B, Varnagy A, Bodis J, Lynch L, O'Farrely C and Szekeres-Bartho J. Commitment of decidual haematopoietic progenitor cells in first trimester pregnancy. 2011 (submitted and recommended for publication in Am. J. Reprod. Immunol.)

## 2. LECTURE AND POSTER PRESENTATIONS

INTERNATIONAL CONFERENCE

**1. Szereday L**, Meggyes M, Lammel K, Barakonyi A, Miko E. The mechanisms of immunological tolerance during pregnancy: the possible role of regulatory T cells in the pathogenesis of pre-eclampsia. The Second International Conference on Regulatory T cells and Th17 Cells and Clinical Application in Human Diseases. Shanghai, China July 17-20, 2010

NATIONAL CONFERENCE

**1.** Meggyes Mátyás, Barakonyi Alíz, Mikó Éva, Mánfai Zoltán, Wilhelm Ferenc, Várnagy Ákos, Bódis József, Illés Zsolt, Júlia Szekeres-Barthó, **Szereday László** Természetes ölősejtek (NK) összehasonlító vizsgálata sikeres vagy sikertelen mesterséges megtermékenyítésen átesett nőkben. A Magyar immunológiai Társaság XXXIX. Vándorgyűlése, Szeged, 2010. November 3-5. Immunológiai Szemle, 2010, 4: 18 (Előadás29)

**2.** Mikó Éva, **Szereday László**, Meggyes Mátyás, Barakonyi Alíz, Farkas Bálint, Bódis József, Szekeres-Barthó Júlia Deciduális haematopoieticus őssejtek jellemzése a terhesség első trimeszterében**.** A Magyar immunológiai Társaság XXXIX. Vándorgyűlése, Szeged, 2010. November 3-5. Immunológiai Szemle, 2010, 4: 17 (Előadás28)

**3.** Barakonyi Aliz, Mikó Éva, Varga Péter, Szekeres-Barthó Júlia, **Szereday László** CD160 NK-sejt-aktiváló receptor pozitív sejtek a perifériás vérben egészséges terhesség különböző szakaszaiban. A Magyar immunológiai Társaság XXXIX. Vándorgyűlése, Szeged, 2010. November 3-5. Immunológiai Szemle, 2010, 4: 50 (Poszter-Klin8)

**4.** Barakonyi Aliz, Mikó Éva, Varga Péter, Farkas Bálint, Bódis József, Szekeres-Barthó Júlia, **Szereday László** Deciduális gamma/delta-T-sejtek jellegzetességei egészséges terhesség során. A Magyar immunológiai Társaság XXXIX. Vándorgyűlése, Szeged, 2010. November 3-5. Immunológiai Szemle, 2010, 4: 51 (Poszter-Klin10)

## 3. RESULTS OF HUMAN EXPERIMENTS

We are confronted with a two-tiered regulatory system: first, effects occur at a **local level** during direct contact of maternal and fetal cells in the placenta, principally through cellular contacts; secondly, effects occur on the **whole maternal immune system**. We had planned to conduct our research in two areas: first investigating the immunological mechanisms in the periphery analyzing peripheral blood mononuclear cells (PBMC) obtained from heparanized venous blood. The other part of the work focused on the local immunological regulatory mechanisms directly investigated in placental samples (decidua and trophoblast).

### 3.1. Investigating the immunological mechanisms of implantation failure after in vitro fertilization (IVF)

In the first part, we started the work on human models investigating the immunological mechanisms in the periphery during implantation in in vitro fertilization (IVF) patients. Assisted reproductive techniques are now routine gynecological procedures. Although their efficiency is undoubted, a notable proportion of IVF cycles result in failed conception resulting in emotional and financial strain for the couples. Much effort has been made to predict IVF treatment

outcome and characterize IVF patients' chances prior to the procedure. A major field of interest is immune profile analysis of IVF candidates since unexplained female infertility, as well as recurrent spontaneous abortion and implantation failure, are thought to be associated with pathological maternal immunotolerance mechanisms.

19 women undergoing IVF treatment cycles were investigated. All women in both groups received the same hormonal supplementation. IVF cycles were successful in 8 patients (sIVF), defined by positive blood β-HCG test performed on the first and third week after embryo transfer followed by live birth at term. The failed IVF group (fIVF) was defined by the negative blood β-HCG tests performed on both the first and the third week after embryo transfer (11 women).

**Patients' demographic, gynecological and treatment characteristics. (Table I.)**

| | **Successful IVF (sIVF)** | **Failed IVF (fIVF)** | P-value |
|---|---|---|---|
| No. of patients | 8 | 11 | |
| Age (years) (mean) | 36,87 | 35,18 | NS |
| Tubal factor (%) | 27,27 | 22,5 | NS |
| Male factor (%) | 45,45 | 62,5 | NS |
| Other (%) | 27,27 | 25 | NS |
| Duration of infertility (years) (mean) | 6,06 | 4,95 | NS |
| Basal FSH levels (IU/ml) | 7,63 | 6,29 | NS |
| Mean no. of previous failed IVF attempts | 2,37 | 1,45 | NS |
| Mean no. of previous miscarriages | 0 | 0 | NS |
| No. of oocytes collected (mean) | 6,6 | 9,63 | NS |
| No. of available embryos for IVF (mean) | 4,62 | 4,45 | NS |
| No. of available embryos for transfer (mean) | 2,87 | 2,45 | NS |
| Gestational age at birth (mean) | 36,75 weeks | - | - |
| Birth weight (mean) | 3060 g | - | - |
| Number of live birth / number of embryos transferred | 8/23 | 0/24 | - |
| Live birth/ patients | 8/8 | 0/11 | P<0.05 |

In summary of that work, we focused on the immunprofile analysis of IVF candidates. Previous studies on peripheral NK cell characteristics of IVF patients were limited to the comparison of blood samples taken prior to the IVF procedure.

In our study we analyzed IVF patient's data from a different point of view. Besides separating successful IVF patients from patients with failed conception, we performed **a follow-up study** and compared patients' data on the day of the oocyte retrieval with the data one week after embryo transfer.

We found different changes of NK and NKT-like cell characteristics in this interval in pregnant women and in non pregnant women. At the second time of investigation (one week after IVF) women with failed IVF showed elevated peripheral NK (both CD56bright and CD56dim) and NKT-like cell ratios, increased perforin containing CD56bright cells, more activated and degranulated NKdim cells and enhanced NK cell activating receptor (CD160, NKG2D) expression on both cell types as well as on both NK cells subsets (Table II. and III.).

**Natural killer cell phenotype characteristics in women with successful conception and in women with failed IVF. (Table II.)**

| | **Successful IVF (sIVF)** | | | **Failed IVF (fIVF)** | | |
|---|---|---|---|---|---|---|
| | **Before** IVF | **After** IVF | P-value | **Before** IVF | **After** IVF | P-value |
| *No. of patients* | *8* | | | *11* | | |
| $CD3^-CD56^+$ cells | 11,09±3,16 | 11,01±2,8 | NS | 5,28±0,74 | 9,66±1,11 | **0,002** |
| $CD56^{bright}$ | 1,41±0,42 | 1,67±0,56 | NS | 0,47±0,07 | 1,26±0,39 | **0,04** |
| $CD56^{dim}$ | 9,94±2,85 | 9,61±2,38 | NS | 4,88±0,73 | 8,53±0,88 | **0,001** |
| CD 160 expression by $CD3^-CD56^+$ cells | 26,31±4,43 | 24,68±5,69 | NS | 20,43±3,38 | 27,71±2,88 | **0,03** |
| $CD56^{bright}$ | 9,58±1,64 | 10,22±3,49 | NS | 6,51±1,52 | 14,9±3,35 | **0,01** |
| $CD56^{dim}$ | 28,14±4,69 | 26,04±6,11 | NS | 21,25±3,51 | 28,61±2,84 | **0,05** |
| NKG2D expression by $CD3^-CD56^+$ cells | 73,7±9,12 | 69,93±8,6 | NS | 50,14±8,35 | 71,64±9,28 | **0,01** |
| $CD56^{bright}$ | 84,66±3,63 | 78,01±6,81 | NS | 73,1±7,51 | 79,91±7,15 | **0,03** |
| $CD56^{dim}$ | 71,77±9,38 | 68,27±9,07 | NS | 47,81±8,16 | 70,71±9,38 | **0,01** |

We assume that one of the **crucial points in failed IVF treatment is the significant increase in NK cells** from the date of oocyte collection to the time one week after embryo transfer in the same individual. All these findings in the fIVF group reflect unfavorable, **Th1 oriented changes** of NK and NKT-like cells during this short period of time around IVF procedure. Comparing the follow-up data of pregnant women they remain principally constant.

**Natural killer cell functional characteristics in women with successful conception and in women with failed IVF. (Table III.)**

| | **Successful IVF (sIVF)** | | | **Failed IVF (fIVF)** | | |
|---|---|---|---|---|---|---|
| | **Before** IVF | **After** IVF | P-value | **Before** IVF | **After** IVF | P-value |
| *No. of patients* | *8* | | - | *11* | | - |
| Perforin expression by $CD3^-$ $CD56^+$ cells | 52,54±6,53 | 50,05±4,96 | NS | 55,59±4,16 | 57,51±3,23 | NS |
| $CD56^{bright}$ | 16,75±3,92 | 18,51±5,21 | NS | 15,29±5,24 | 24,34±5,49 | **0,02** |
| $CD56^{dim}$ | 55,51±7,02 | 52,41±5,25 | NS | 58,44±4,08 | 60±3,34 | NS |
| *No. of patients* | *7* | | - | *9* | | - |
| CD107a expression by $CD3^-$ $CD56^+$cells | 15,69±2,6 | 15,27±3,8 | NS | 12,48±2,23 | 15,94±2,57 | **0,02** |
| $CD56^{bright}$ | 8,73±2,85 | 10,03±2,48 | NS | 12,97±2,96 | 13,95±3,85 | NS |
| $CD56^{dim}$ | 16,73±2,64 | 15,68±3,96 | NS | 12,01±2,49 | 15,92±2,52 | **0,02** |

Previous studies on peripheral NK cell characteristics of IVF patients were limited to the comparison of pregnant and non pregnant patients' data from one blood sample prior to the IVF procedure. Here we demonstrate that changes of NK and NKT-like cell phenotype during the implantation window and early pregnancy in the same individual may also be important for successful embryo implantation.

### 3.2. Investigating the expression of PACAP in the human placenta and its possible effect on the survival of choriocarcinoma cells

We have also continued good progress investigating different regulatory effects of a neuropeptide thought to play a role in reproductive functions, such as pregnancy. Pituitary adenylate cyclase activating polypeptide (PACAP), a neuropeptide with survival-promoting actions, has been shown in endocrine organs and is thought to play a role during pregnancy. In the present study, we determined the concentrations of PACAP in first trimester and full-term human placentas using radioimmunoassay.

We found high levels of PACAP38 and lower levels of PACAP27 in different parts of the full-term human placenta. PACAP38 content increased in the placenta during pregnancy, both on the maternal and the fetal side (Fig. 1.).

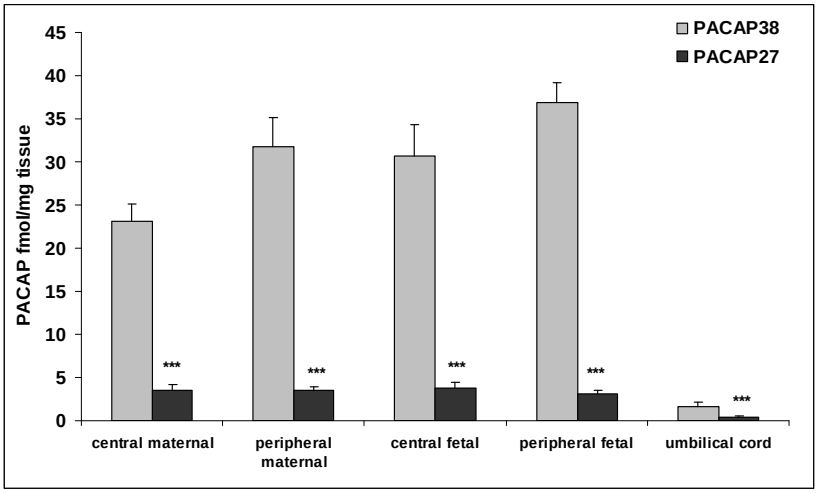


Fig 1. PACAP38 and PACAP27 concentrations as measured by RIA in different parts of the mature human placenta and umbilical cord.

Choriocarcinoma cells have been previously shown to have functional receptors for PACAP and to react with increased cAMP levels upon PACAP treatment. The effects of PACAP on the survival of human choriocarcinoma cells was investigated using flow cytometry and MTT cell viability asssay in cells exposed to the widely-used chemotherapeutic agent, methotrexate. It was found that PACAP neither influenced the survival of cytotrophoblast cells nor affected the cellular response to the death-inducing effect of the chemotherapeutic agent methotrexate (Fig. 2.).

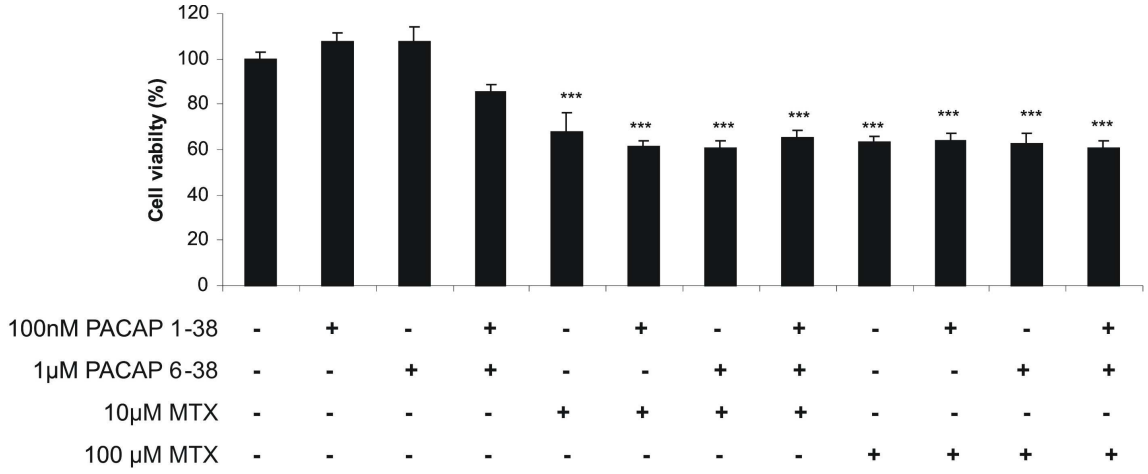


Fig. 2. Viability of JAR human choriocarcinoma cells as measured by MTT assay.

The present observations further support the significance of PACAP in the human placenta. The observation that PACAP did not influence the effects of methotrexate may have future clinical importance, showing that PACAP does not decrease the effects of certain chemotherapeutic agents.

### 3.3. Analysis of the expression profiles of peripheral CD160+ lymphocytes during the course of healthy human pregnancy

In our other paper published in the American Journal of Reproductive Immunology, we investigated the phenotype of peripheral CD160+ cells during healthy pregnancy.

Sixty-one pregnant women at different stages of pregnancy without any risk factors and 12 age-matched non pregnant healthy women were included in this study. In order to analyze the whole course of healthy pregnancy we divided the 40 gestational weeks into 4 stages: into 3 trimesters as routinely and additionally we defined the last 4 gestational weeks as "late pregnancy" (Table IV).

**Comparison of patients' characteristics (Table IV.)**

| **Groups** | **Non-pregnant** | **1st trimester** | **2nd trimester** | **3rd trimester** | **Late pregnancy** | **Significance** |
|---|---|---|---|---|---|---|
| **Age** (year) | 31.00 ±*3.09* | 32.12 ±*3.22* | 26,63 ±*2.05* | 28.36 ±*1.17* | 31,25 ±*1.77* | NS * |
| **Gestational age** (weeks) | - | 8.4 (6-12) | 16.1 (16-17) | 26.7 (25-29) | 38.6 (37-41) | - |
| **No. of patients** (*n*) | *12* | *22* | *12* | *17* | *10* | - |

CD160 is one of the NK cell receptors which could deliver activating signals regulating cytotoxicity and cytokine production. During this work we investigated the systemic CD160 receptor expression profile providing new data about the nature of CD160+ lymphocytes at different stages of normal pregnancy.

Studying the peripheral blood of healthy pregnant and non-pregnant women we found that CD160 receptor is present on peripheral lymphocytes during the whole course of gestation (Fig. 3a.). During the course of pregnancy CD160+ lymphocytes showed a significant increase of the early T cell activation marker (CD69) expression in the 3rd trimester which was followed by a significant fall in the late pregnancy stage (Fig. 3b.). Our data revealed that peripheral CD160 receptor could be functional rather in the 3rd trimester than in other stages.

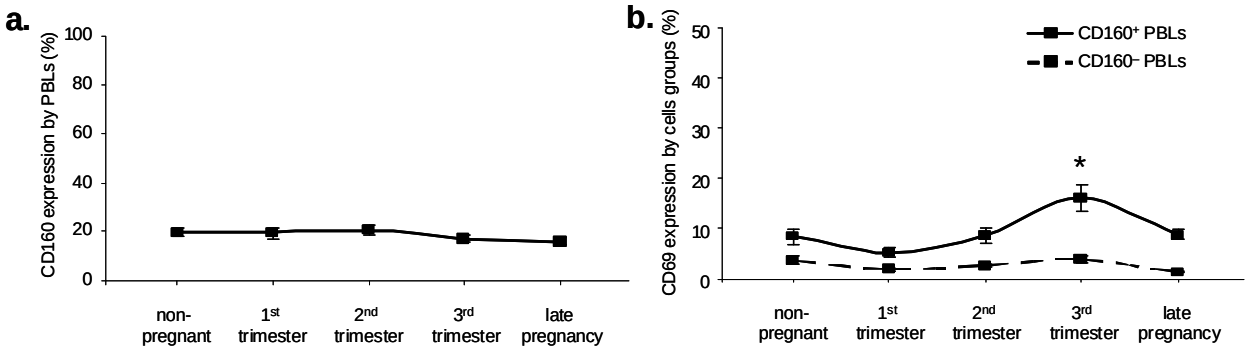


Fig. 3. a). The expression of CD160 NK cell activating receptor by peripheral blood lymphocytes of healthy pregnant and non-pregnant women. b).Presence of CD69 activation marker on $CD160^+$ and $CD160^-$ lymphocytes of healthy pregnant and non-pregnant women.

We also analyzed two other NK cell receptors on peripheral blood lymphocytes (PBLs): the NK cell activating NKG2D receptor and the NK cell inhibitory NKG2A one. While NKG2A expression (Fig. 4a.) was constant throughout pregnancy, NKG2D showed a strong significant decrease in the 1st trimester followed by a significant increase in the 2nd trimester (Fig. 4b.). It could be possible that NKG2D receptor activation is useful during the sentinel function of NK or γ/δ T cells in the latter stages of healthy pregnancy, for example against infectious agents. NKG2A inhibitory receptor on PBLs showed a constantly low expression in all patient groups investigated.

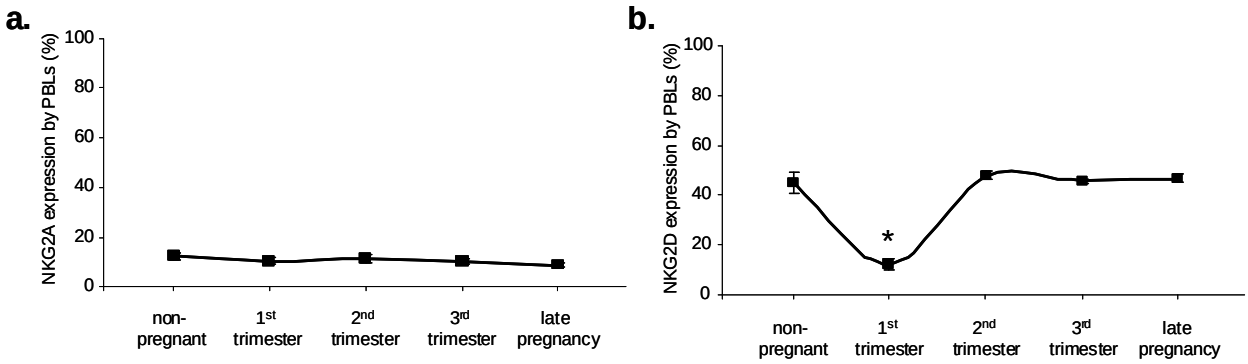


Fig. 4. The expression of NKG2A NK cell inhibitory and NKG2D activating receptor by peripheral blood lymphocytes of healthy pregnant and non-pregnant women.

Furthermore, in our study we described some basic characteristics of CD160 expressing lymphocytes which are independent from pregnancy or its stage. Accordingly, peripheral CD160+ cells show a high NK activating (Fig. 5a.) and inhibitory (Fig. 5b.) receptor expression, an elevated activation rate and Vδ2+ T cells have a special character to express CD160 receptor. The NKG2D and the NKG2A profile of CD160+ lymphocytes showed a strong significant fall during the period of implantation compared to non pregnant group, which suggests that other receptor functions could be involved in the control of CD160+ cells during that time.

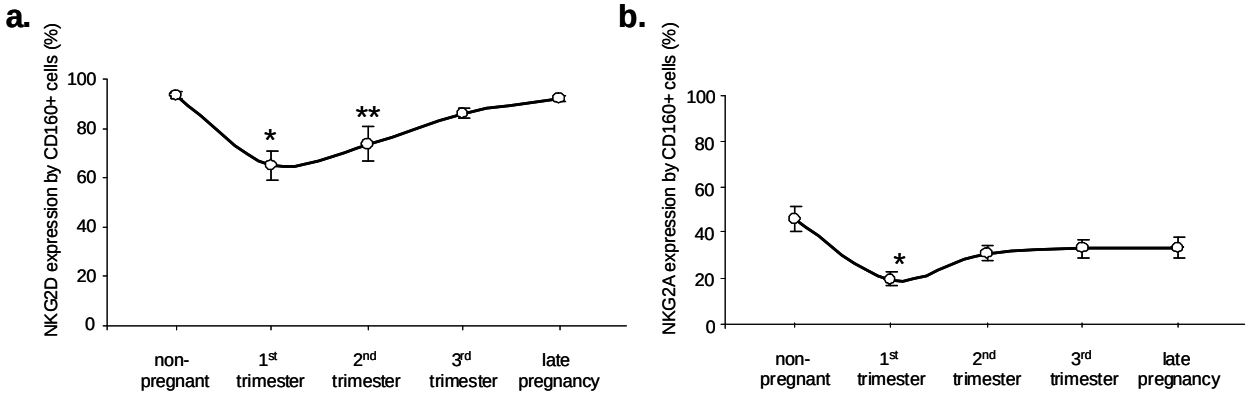


Fig. 5. The expression of activating NKG2D and inhibitory NKG2A NK cell receptor on peripheral $CD160^+$ lymphocytes during the course of healthy pregnancy.

In the 2nd trimester, 20% of peripheral $CD160^+$ cells express TCR γ/δ, which are mostly Vδ2+, suggesting that Vδ2 receptor could be of interest on $CD160^+$ lymphocytes (Fig. 6a.). There is a low percent of $CD160^+$ cells which are Vδ2 TCR+ during implantation followed by an apparent but not significant augmentation in the 2nd trimester (Fig. 6b.). Moreover, in the 2nd trimester more than 65% of Vδ2+ T cells bear CD160 receptor conserving this high expression level until the end of pregnancy. It suggests that this γ/δ T lymphocyte population could have

immunological significance in CD160 receptor function from that time on. This hypothesis is supported by our observation that only 19% of Vδ2-negative lymphocytes express CD160 in the 2nd trimester and there are no biologically relevant changes in other stages.

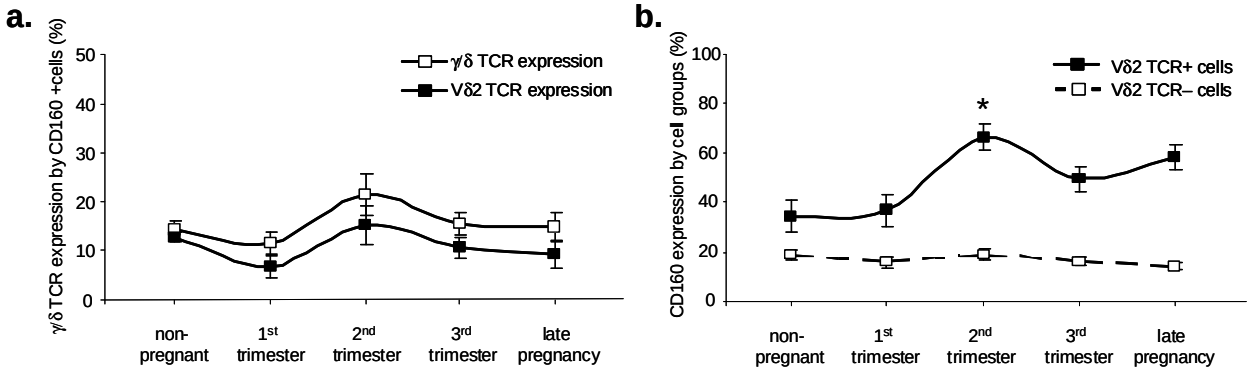


Fig. 6. Gamma/delta TCR expression on CD160+ peripheral blood lymphocytes during the course of pregnancy.

Our results revealed that CD160+ cells could be able to produce pro-inflammatory cytokines which could play a part in the controlled sterile inflammatory response characterizing normal human gestation assisting the maintenance of healthy pregnancy.

### 3.4. Phenotypic characterization of decidual haematopoietic progenitor cells in first trimester in healthy pregnant women and in women with early miscarriage.

In our paper forthcoming in American Journal of Reproductive Immunology, we demonstrated the presence of haematopoietic progenitor cells (HPC) in first trimester human deciduas. Decidual lymphocytes play a major role in the recognition of the semi-allogenic fetus creating a favorable environment for implantation, placentation and for the early development of the fetus. This recognition of fetal HLA antigens presented by the extravillous trophoblast leads to the control of trophoblast invasion. Abnormal implantation is associated with many pregnancy related disorders. Little is known about the homing, proliferation and differentiation of decidual lymphocytes. Besides being recruited from the periphery, local (extrathymic) lymphocyte development is also conceivable.

Eight pregnant women with early (7–12 weeks) miscarriage of unexplained etiology (mean age of 34.87 ± 1.76 range, 30-45 years) were recruited to participate in the study and served as a pathological pregnant group. For a relevant control group, we selected 16 healthy women who underwent elective terminations of apparently normal pregnancies at the 6-10 weeks of gestation. Neither had a history of spontaneous abortion, ectopic pregnancy, preterm delivery, or stillbirth. The mean age of these women was 34.69 ± 2.62 (range, 24-47) years.

Direct evidence for the presence of heamatopoietic progenitor cells in the adult endometrium was obtained by flow cytometric phenotypic analysis of isolated endometrial lymphocytes by Lynch et. al.. HPCs were identified by their co-expression of CD34 and the leukocyte marker CD45.

Identification of decidual HPC commitment was based on the expression of differentiation markers on CD34+CD45+ cells. CD7 receptor is an early marker for lymphoid HPC development including both T cell and NK cell differentiation potential. CD122 receptor is essential for NKT cell 17 and CD127 for γ/δ T cells development.

Haematopoietic progenitor cells were identified by their co-expression of CD34 and the leukocyte marker CD45 by flow cytometric analysis in all decidual samples of both healthy pregnant women (0.44%) and in patients with miscarriage (0.48%) (Fig. 7.). Compared to peripheral blood, we found a significant increase in the percentage of decidual HPCs within the lymphogate in both healthy pregnant women and women with spontaneous abortion (0.03 vs 0.44, $p<0.02$ and 0.01 vs. 0.48, $p<0.01$ respectively) (Fig. 7.).

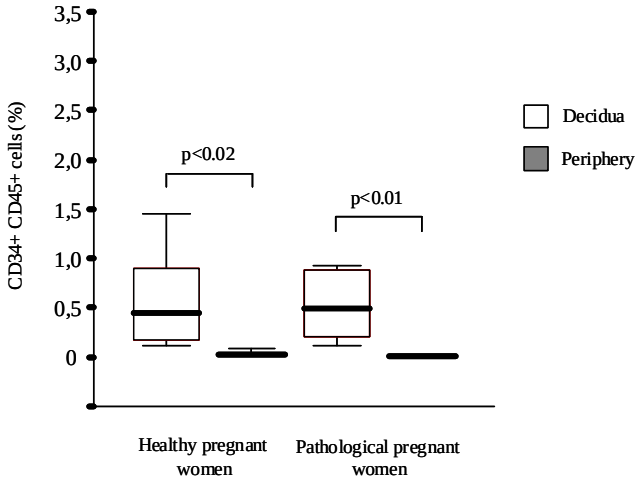


Fig. 7. Phenotypic detection of haematopoietic progenitor cells in peripheral blood and in the decidua

C-kit (CD117) is a cytokine receptor for the cytokine stem cell factor (CSF) and it is expressed on the surface of the HPC in the early phase of differentiation. C-kit expression of CD34+CD45+ HPCs was lower in the decidua compared to the periphery in both investigated groups but the difference did not reach the level of statistical significance (Fig. 8A.).

For the analysis of activation and differentiation potential of HPCs we determined the expression of CD45RA on CD34+CD45+ HPCs. In healthy pregnant women there is a significant decrease (36.81 vs 58.73, $p<0.01$) in CD45RA expressing HPCs in the decidua when compared to the periphery (Fig. 8B). There was no significant difference in the expression of CD117 and CD45RA by HPCs in the decidua between healthy and pathologic pregnant women.

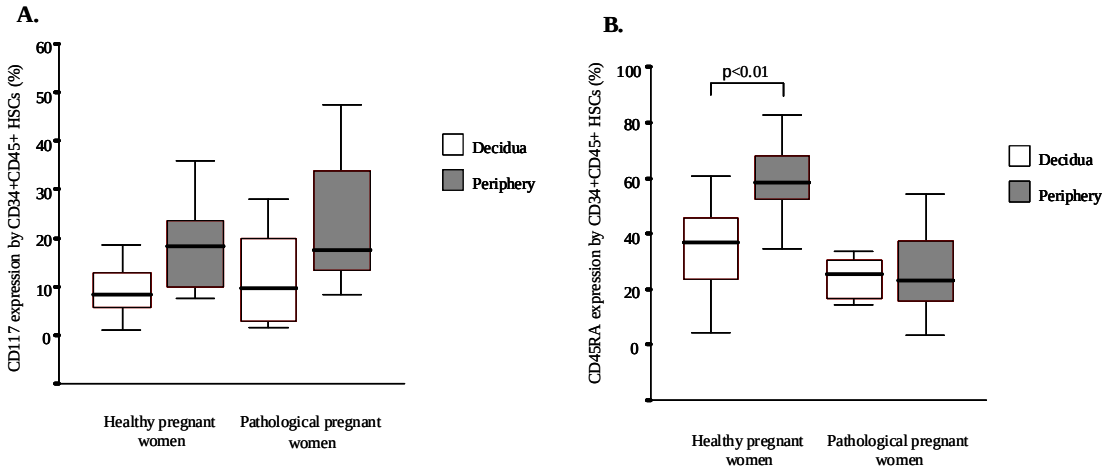


Fig. 8. CD117 (c-kit) and CD45RA expression by CD34+CD45+ HPCs

Henceforward, we focused on the lymphoid differentiation markers since decidual lymphocytes play a crucial role in the maintenance of early pregnancy.

T/NK progenitor cells were identified by their expression of CD7 on CD34+CD45+ cells. Among decidual HPCs, T/NK precursor levels are significantly increased in the decidua compared to peripheral blood in both healthy and pathologic pregnancy (Fig. 9A).

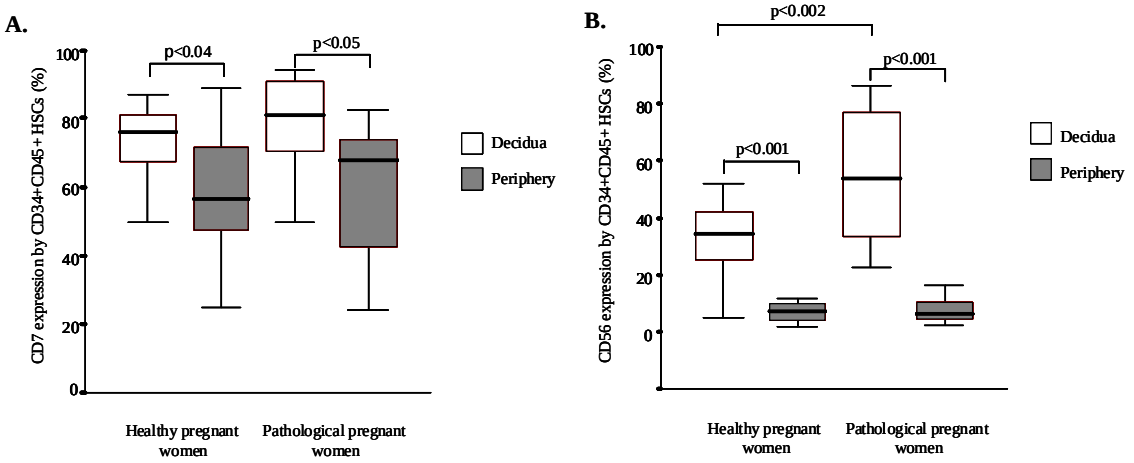


Fig. 9. The expression of CD7 and CD56 by CD34+CD45+ HPCs in the decidua and in matched blood of healthy pregnant and pathological pregnant women.

Both in the healthy pregnant and in the pathologic pregnant group, there are significantly higher decidual CD56+CD34+CD45+ NK progenitor populations than in the peripheral blood (Fig. 9B.; healthy pregnant: 34.48 vs. 7.41, $p<0.001$; pathologic pregnant: 54.00 vs. 6.15, $p<0.001$). Furthermore, among HPCs in women with spontaneous abortion, there is a significantly larger NK progenitor cell population in the decidua compared with healthy pregnancy (Fig. 9B and 4D; 54.00 vs. 34.48, $p<0.002$)

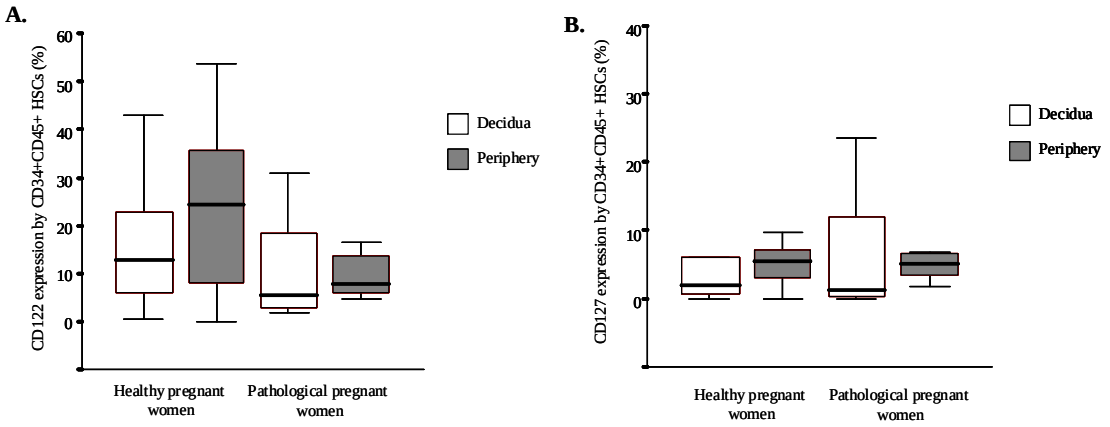


Fig. 10. The expression of CD122 and CD127 by CD34+CD45+ HPCs in the decidua and in matched blood of healthy pregnant and pathological pregnant women.

NKT progenitors (CD122+CD34+CD45+ HPCs) and γ/δ T cell progenitors (CD127+CD34+CD45+ co-expression) were present in the decidua and their occurrence shows similar distribution as seen in the periphery in both donor group (NKT progenitors: Fig. 10C, 12.86 vs. 24.14 and 5.51 vs.7.85; γ/δ T cell progenitors: Fig 10D. 2.05 vs. 5.43 and 1.06 vs. 4,94).

In this work we demonstrated the presence of HPCs in first trimester deciduas. Apart from a small proportion of immature, c-kit expressing HPCs the majority of decidual haematopoietic progenitor cells appear to be already committed to lineage specific proliferation.

Here we demonstrated the decidual presence of the T/NK, NK, NKT and γ/δ T cell progenitors suggesting local autonomous self-support of the decidual lymphocyte pool. Additionally, the decidual lineage-committed lymphoid cell population shows a similar distribution profile to mature decidual lymphocytes, suggesting they may arise locally. Compared to peripheral blood, the ratio of the T/NK precursor cells and NK precursors among decidual HPCs is significantly higher in the decidua. The observations that both precursor cell types, the less committed T/NK progenitors and the more differentiated NK progenitor cells are enriched in the decidua strongly suggest that the dominance of uterine NK cells is ensured from the beginning of local lymphocyte development.

In this study we identified decidual HPCs in women with early spontaneous abortion and the levels of decidual CD56+ progenitor cells among HPCs was found to be significantly higher compared to their levels in decidual samples of healthy pregnant women. These data suggest a dysregulation of decidual HPC maturation resulting in an increased density of NK cells at the feto-maternal interface. High numbers of decidual NK cells may at least partly account for disturbances in the immunological recognition of the fetus which is strongly related to the success of implantation and placentation via different effector functions, such as cytokine production. HPC dysregulation may pose a new target area for reproductive immunotherapeutic approaches, such as the treatment of infertility or habitual abortions and the practical significance of local decidual haematopoiesis will further increase.

## 4. RESULTS OF ANIMAL EXPERIMENTS

Animal models are important, as they allow for controlled experiments and analysis of multiple time-points during pregnancy.

The mouse gene family of TIM molecules includes 8 members (encoding TIM-1, TIM-2, TIM-3 and TIM-4 proteins and the putative TIM-5 to TIM-8 proteins), while the human gene family includes 3 members encoding TIM-1, TIM-3 and TIM-4 proteins.

TIM-3 was the first, and is presently the only, surface molecule that can specifically identify Th1 cells in both mice and humans. Engagement of TIM-3 by its ligand galectin-9 negatively regulates IFN-γ secretion and influences the ability to induce T cell tolerance in both mice and humans.

Analyzing lymphocytes from healthy pregnant mice by flow cytometry, we found an elevated TIM-3 expression on different cell populations (CD8, CD4, γ/δ T cells) in the decidua compared to peripheral splenocytes.

Galectin-9 (the ligand for TIM-3) expression by spongiotrophoblast was successfully detected at the feto-maternal site of mouse (Balb/c mice) placenta by immuno-histochemistry.

Since we could detect galectin-9 expression by healthy mouse placentae, we analyzed the gene expression of galectin-9 by real-time RT-PCR during healthy pregnancy investigating healthy placentae and fetal resorptions. In fetal resorptions examined during mid-pregnancy, galectin-9 expression is 4-times lower compared to healthy mouse placentae, suggesting the role of TIM-3/galectin-9 pathway in the pathomechanism of pathologic pregnancies.