Article

Higher interleukin-18 and mannose-binding lectin are present in uterine lumen of patients with unexplained infertility

Dr Nathalie Lédée received her science PhD in 2003 and her MD in obstetrics and gynaecology in 1993 from the University Paris VI, France. She is assistant professor in Reproductive Medicine at the University Versailles St. Quentin en Yvelines. From 2004 to 2008, she was work-package leader in the European network EMBIC focusing on embryo implantation (Embryo Implantation Control). Her main field of research is uterine receptivity and involvement of cytokines/chemokines in the process of embryo implantation.

Dr Nathalie Lédée

P Oger[1], R Bulla[2], F Tedesco[2], A Portier[3], S Dubanchet[3], M Bailly[1], R Wainer[1], G Chaouat[3], N Lédée[3,4]
[1]Departments of Obstetrics and Gynecology and Reproductive Medicine, Centre Hospitalier Intercommunal Poissy St.-Germain en Laye, University UVSQ UPRESS EA 2493, 78300-F Poissy, France; [2]Department of Physiology and Pathology, University of Trieste, via Fleming 22, 34127 Trieste, Italy; [3]INSERM, U782, University Paris-Sud, UMR-S0782, F-92140 Clamart, France
[4]Correspondence: e-mail: nathalie-ledee@orange.fr

Abstract

The uterine luminal environment was explored with regard to interleukin-18 (IL-18) and mannose-binding lectin (MBL) and the possibility that the procedure of flushing the uterine cavity would optimize the physiological initial pseudo-inflammatory uterine reaction. Uterine flushings were performed among 175 IVF/intracytoplasmic sperm injection (ICSI) patients at the time of oocyte retrieval and the cycles were compared with a control group matched for age, number of previous attempts and type of assisted reproductive procedure (IVF or ICSI) in which no flushing were performed ($n = 175$). Samples collected were divided into two groups according to the presence/absence of endometrial cells in samples. IL-18 and MBL expressions were explored by enzyme-linked immunosorbent assay. Implantation rates were significantly higher in those patients who underwent the uterine flushing compared with controls ($P = 0.04$). Luminal concentrations of IL-18 and MBL were higher if endometrial cells were present in flushings, suggesting endometrial origin of the secretion. Both concentrations of MBL and IL-18 were higher in patients with unexplained infertility compared with patients involved in IVF/ICSI for male or tubal infertility ($P = 0.005$ and 0.02, respectively). The exploration of the endoluminal environment before oocyte retrieval may enhance pregnancy rates and show distinct features in patients with unexplained infertility.

Keywords: *embryo transfer, endoluminal uterine fluid, interleukin-18, IVF, mannose-binding lectin, uterine flushing*

Introduction

Implantation failure is the most frequent event occurring after embryo transfer and may be related to an inadequate uterine receptivity in some cases (Diedrich *et al.*, 2007). A wide variety of uterine functions, as well as some facets of the embryo development, appear to be controlled by locally secreted cytokines which probably play a major role in the development of such an adequate uterine receptivity (Giudice, 1994; Chard, 1995; Chaouat, 1995; Simon *et al.*, 1998). Together they define a complex network, whose adequate may permit a successful implantation and, later on, promote optimal placental growth and function. Conversely it may cause placental dysfunction, abnormal uterine development, and eventually a real immune rejection of the fetal–placental unit leading to early pregnancy loss/abortion.

The preparation of the uterus to be receptive has been described as an initial pseudo pro-inflammatory reaction allowing the attraction of innate immunity competent immune cells (Mor, 2008). This first reaction is followed by an 'armed anti-inflammatory' reaction at the time of uterine receptivity characterized by a depletion of

macrophages, B lymphocytes and T-CD8 cells which are replaced by an inflow of CD56 bright cells, regulatory T cells and dendritic cells (Noun *et al.*, 1989; Wood *et al.*, 1997). The present study first explored the possibility that, by the action of the flushing procedure itself, one would eventually trigger/optimize the initial pseudo-inflammatory reaction at the time of oocyte retrieval, 1 week before the uterus finally reaches the stage of uterine receptivity.

The uterine endometrial epithelial cells undergo profound changes in structure and function in preparation for blastocyst implantation. Epithelial cells have a structural and functional polarized orientation as well as a polarized secretion over the cycle (Fahey *et al.*, 2005). In mice, some authors described the influence of the polarization on the subsequent blastocyst development (Azadbakht *et al.*, 2007).

It was postulated that the technique of uterine flushing would allow exploration of the apical uterine epithelial cell secretion. A previous study described that interleukin-18 (IL-18) detection in the luminal environment at the time of oocyte retrieval was associated with a poor outcome suggesting that more investigations were required to document the immunological composition of luminal environment at the time of oocyte retrieval (Ledee-Bataille *et al.*, 2004). IL-18 appears as a bivalent cytokine regarding the implantation process itself, i.e. possibly deleterious for implantation by its well-known action as an γ-interferon-inducing factor (Okamura *et al.*, 1995; Ushio *et al.*, 1996; Torigoe *et al.*, 1997). In contrast, at the mild luteal phase during the implantation window, IL-18 seems to be necessary for implantation because of its angiogenic properties (Park *et al.*, 2001) especially in regard to spiral artery remodelling (Xie *et al.*, 2005).

Concerning the time of oocyte retrieval, thus about 6 days before implantation, the study mainly explored the possible negative role of IL-18 as a γ-interferon inducer. The hypothesis was that IL-18 secretion in the luminal compartment reflects an underlying endometrial activation of the IL-18 system. IL-18 is synthesized in the endometrium as a biologically inactive precursor (Yoshino *et al.*, 2001), a 24-kDa molecule, which requires cleavage by IL-1β converting enzyme (ICE, also known as caspase-1) to generate the biologically active 18 kDa monomer able to be secreted (Ghayur *et al.*, 1997; Gu *et al.*, 1997). Hence its detection in the uterine luminal environment by uterine flushing is associated with its activation. IL-18 stimulates the production of IL-1β, which exerts pro-inflammatory effects through the IL-18 receptor, which is identical to IL-1 receptor–binding protein (Lebel-Binay *et al.*, 2000).

Mannose-binding lectin (MBL) is a complement factor mainly produced by the liver that binds to carbohydrate ligands and activates the MBL-associated serine proteases (MASPs) leading to the triggering of the lectin pathway (Fujita *et al.*, 2004; Thiel, 2007). The serum concentration of MBL has been found to increase early in pregnancy during the first trimester and is associated with a significant increase in MBL pathway activity (van de Geijn *et al.*, 2007). Reduced maternal MBL concentrations have also been described as a risk factor for preterm birth and reduced birthweight (Annells *et al.*, 2004). The presence of MBL in the uterine environment has not been investigated nor is clear whether the local concentration is correlated with a successful implantation.

Therefore, a matched controlled retrospective study was conducted to explore: (i) the effect of the method of uterine flushing on pregnancy rates; and (ii) a prospective documentation of IL-18 and MBL concentrations in the luminal environment on a large cohort of patients.

Materials and methods

Patients

From March 2004 to March 2006, 175 patients underwent uterine flushing immediately before oocyte retrieval and after the local anaesthesia. The only inclusion criteria applied was oocyte retrieval under 41 years of age. All the patients were fully informed and the institutional review board approved this investigation (CCPPRB, protocol 01–78).

For the same time period, a control group of 175 patients was selected who were matched on age, number of previous attempts and type of assisted reproduction technique (IVF or ICSI) and who did not undergo uterine flushing (**Table 1**).

The study defined categories of analysis for aetiologies, responses to the stimulation and patterns of endometrium. Male infertility was defined classically by the sperm parameters. The following definitions for analysis of female infertility were applied: (i) unexplained infertility: defined by ovulatory cycles, normal semen parameters, normal hysterosalpingography and hysteroscopy, normal ovarian reserve and a conception period stretching over 2 years, no pregnancy after ovarian induction cycles followed most of the time by intrauterine inseminations; (ii) ovarian infertility: defined by dysovulation or anovulation without other abnormalities (sperm, tubes, uterus, ovarian reserve), no pregnancy after ovarian induction and ovarian induction followed by intrauterine inseminations; (iii) low ovarian reserve infertility: defined by FSH over 10 mIU/ml on day 3 and/or antral follicles count below five antral follicles assessed by ultrasound on day 3; (iv) endometriosis: diagnosed by laparoscopy and/or nuclear magnetic resonance imaging; (v) recurrent abortions or IVF/ICSI for genetic diseases; and (vi) fertilization failure.

Response to ovarian stimulation were classified in three categories: (i) low responders (oestradiol below 1200 pg/ml on the day ovulation was triggered); (ii) mild responders (oestradiol between 1200 and 3000 pg/ml) and high responders (oestradiol over 3000 pg/ml).

The endometrial pattern was evaluated on the day of human chorionic gonadotrophin administration by vaginal ultrasound, and was classified as type I if a triple-line multilayer was observed and as type II when it was fully