# Prolactin and proinflammatory cytokine expression at the fetomaternal interface in first trimester miscarriage

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**Objective:** To investigate the expression of prolactin (PRL), PRL-receptor (PRL-R), and the $T_H1$ cytokines interleukin-2 (IL-2), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and interferon-$\gamma$ (IFN-$\gamma$) at the maternofetal interface.
**Design:** Case-control study.
**Setting:** University hospital unit of gynecology and obstetrics and research laboratories.
**Patient(s):** Women undergoing suction curettage for spontaneous miscarriage (study group) and voluntary termination of pregnancy (control group) in the first trimester.
**Intervention(s):** Samples of decidua and villi collected and histologically examined at the time of suction curettage.
**Main Outcome Measure(s):** Evaluation of all villous samples for karyotype with only euploid cases included; detection of transcripts of PRL, PRL-R, TNF-$\alpha$, IFN-$\gamma$, and IL-2 by qualitative reverse-transcriptase-polymerase chain reaction (RT-PCR); investigation of pattern and site of expression by immunohistochemistry.
**Result(s):** In both groups, PRL-R and IFN-$\gamma$ were broadly expressed. The expression of PRL was impaired or absent in the villi of the study group compared with controls. Expression of TNF-$\alpha$ was reduced, although not statistically significantly, in both decidual and villous samples of the study group. Immunohistochemical analysis showed the lack of IL-2 expression in decidual specimens of the control group versus the full expression shown in the study group.
**Conclusion(s):** Our results highlight the correspondence between PRL expression and vital pregnancy and the involvement of the $T_H1$ cytokines with different specific roles at the implantation site. Prolactin and IL-2 may reciprocally influence expression. (Fertil Steril® 2013;100: 108–15. ©2013 by American Society for Reproductive Medicine.)
**Key Words:** Cytokines, decidua, miscarriage, prolactin, villi

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Embryo implantation is currently considered the limiting factor for the establishment of pregnancy. Up to 60% of all pregnancies are terminated at the end of the peri-implantation period and a further 10% of pregnancies, mostly before 12 weeks of gestation, result in miscarriage; an imbalance in embryo-maternal cross-talk may be the major reason (1, 2). A number of studies have investigated the physiology and pathology of the maternal side, but the embryonic side has not been investigated to the same extent. Cytokines are known to play an important role in embryo-maternal signaling. Cytokines of the embryo-maternal complex are virtually involved in the regulation of all stages of gestation (3) and may be directed from the embryo toward the mother and vice versa. The break in "cytokine balance" induced by exogenous or endogenous factors can lead to spontaneous abortion and even to pregnancy complications (4). In animal studies,

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$T_H1$ immunity has been related to implantation failure and fetal resorption (5, 6), whereas the $T_H2$ cytokines produced at the fetomaternal interface have been demonstrated to be beneficial to the maintenance of pregnancy through the suppression of cellular cytotoxicity (7).

In humans, the existence of a $T_H2$ bias in the successful pregnancy has not been clearly validated. The update of the $T_H1/T_H2$ paradigm in a $T_H1/T_H2/T_H3$ (8) or $T_H1/T_H2/T_H3$/Treg-1 (9) paradigm stems from the assumption that a $T_H1$ proinflammatory cytokine milieu in the implantation site is potentially hazardous for fetal development. Against the belief that the type of immune reactivity, type 1 or type 2, is strictly predictive of implantation outcome and pregnancy maintenance, some investigators have suggested that reproductive failures cannot be simply related to an inflammatory pathway and that some proinflammatory cytokines are physiologically expressed in healthy pregnancies (10, 11).

Prolactin (PRL) is a peptide hormone essentially secreted by the anterior pituitary and, to a lesser extent, by other extrapituitary tissues (12, 13) such as the endometrium (14–16). Endometrial PRL is synthesized by decidualized endometrial cells in the mid-to-late secretory phase in a nonconception cycle and throughout pregnancy (17). The effects of PRL are mediated by a membrane-bound receptor (PRL-R), member of the superfamily of cytokine receptors. If pregnancy occurs, PRL-R expression is maintained and localized in the decidua, placental trophoblast, and amniotic epithelium (18). The coordinated temporal pattern of expression of both PRL and PRL-R in the nonpregnant and pregnant endometrium suggests that PRL may have an important role in the process of implantation, acting as a differentiation factor rather than as mitogenic. The relationship between the proinflammatory $T_H1$ cytokines, classically involved in miscarriage, and PRL is still poorly understood. The demonstration of a dose-dependent inhibition of the synthesis and release of PRL in primary decidual cell cultures by exposure to tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (19), interleukin-2 (IL-2) (20), and interferon-$\gamma$ (IFN-$\gamma$) (21) suggests the presence of a network of molecules strongly interrelated at the maternofetal interface. Our study investigated the expression of PRL, PRL-R, and the $T_H1$ cytokines IL-2, TNF-$\alpha$, and IFN-$\gamma$ at the implantation site in decidua and villous samples collected from women undergoing suction curettage for spontaneous abortion (study group) and voluntary termination of pregnancy (control group).

## MATERIALS AND METHODS

The study was performed at the units of Obstetrics and Gynecology and Human Pathology of the San Paolo Department of Health Sciences in Milan in collaboration with the Laboratory of Endocrine and Metabolic Research of the Istituto Auxologico Italiano. The study protocol was approved by the ethics committee of San Paolo Hospital, and informed consent was given by all study participants.

### Patient Selection

One hundred and twelve patients aged 16–41 years (median age 31.4) undergoing suction curettage in the first trimester of pregnancy ($\leq$13 weeks of amenorrhea) were recruited: 84 affected by spontaneous miscarriage (SM) and 28 asking for voluntary termination of pregnancy (VTP). The inclusion criteria were history of regular menstrual cycles, spontaneous conception, normal uterine anatomy as demonstrated by transvaginal ultrasonography, and gestational sac and embryo sizes consistent with gestational age. Exclusion criteria were an extrauterine location of the gestational sac, the presence of known causes of miscarriage, and the presence of vaginal bleeding at the time of hospitalization. In the SM group, miscarriage was defined as a fetal pole lacking fetal heart rate activity in women without clinical signs of expulsion; in the VTP, group the fetal heart rate activity was present before surgery.

### Sample Collection

Within 3 hours of surgery, all women were given 1 mg of intravaginal gemeprost (Cervidil; Serono). At the time of suction curettage, the gestational tissues were removed in sterile conditions and macroscopically assessed through several washes in sterile phosphate-buffered saline solution to separate chorionic villi from decidua and embryo. For each patient, a sample of decidua and villi were collected and histologically examined by an experienced gynecologic pathologist; only tissues in agreement with the macroscopic assessment were selected for the study. Tissues collected for the RNA extraction were stored in RNAasi-free test tubes, snap-frozen in dry ice, and stored in liquid nitrogen. The samples collected for immunohistochemical (IHC) analysis were initially stored in formalin. In all cases, the analysis of fetal karyotype was performed using direct preparation of chorionic villi, and only samples with a normal karyotype were used for further analysis.

### Immunohistochemistry

Immunohistochemical studies were carried out on 4 $\mu$m thick tissue sections using a Novolynk Polymer Detection System (Novocastra Laboratories Ltd.) with rabbit anti-human polyclonal prolactin antibody (DakoCytomation), mouse anti-human tumor necrosis factor-$\alpha$ monoclonal antibody (clone 195; Chemicon International), mouse anti-human monoclonal IL-2 antibody (clone 297C16F11, BioSource Europe S.A.) and rabbit anti-human IFN-$\gamma$ polyclonal antibody (sc-8308; Santa Cruz Biotechnology). Sections were deparaffinized in Bio-Clear (Bio Optica) for 20 minutes then washed twice in ethanol.

For the TNF-$\alpha$ analysis, the slides were placed in a water bath containing 9 mM sodium citrate at pH 6.0 for 30 minutes at 95°C. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in distilled water for 10 minutes. Staining was performed with 3,3′ diaminobenzidine (DAB) as a chromogen.

For the PRL staining, the primary antibody was applied at a concentration of 1:500 in 0.5% bovine serum albumin (BSA) and sodium azide and incubated 30 minutes at room temperature. For IL-2 staining, the primary antibody was applied at a concentration of 1:50 in 0.5% BSA and sodium azide and incubated 30 minutes at room temperature. For