INTERNATIONAL CORNER

ENDOMETRIAL FUNCTION AT THE PREIMPLANTATION STAGE IN WOMEN UNDERGOING ASSISTED REPRODUCTION TECHNOLOGY CYCLES

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ABSTRACT

IVF-ET; LIF; PP14; VEGF; endometrial function; uterine artery Doppler velocimetry; ART.
Abstract Objective: To evaluate endometrial function before embryo implantation in women undergoing assisted reproduction technology cycles (ART). Patients: Thirty-five infertile couples, divided into two groups with respect to reproductive outcome: 12 pregnant and 23 not pregnant. Main outcome measures: Serum leukemia inhibitory factor (LIF) , placental protein 14 (PP14), cancer antigen 125 (CA 125) , E2, and P; serum and follicular vascular endothelial growth factor (VEGF); resistance index, pulsatility index, and peak systolic velocity of the uterine, radial and follicular arteries. Results: The two groups were homogeneous for clinical, fertilization and color Doppler data. P, CA 125 and VEGF levels were comparable. E2 levels were significantly decreased (p=0,04) from the day of hCG administration to the day of embryo transfer only in the not-pregnant group (from 1568±552 pg/ml to 1187±653 pg/ml) . Serum P and PP14 levels were significantly increased (p<0,05) from the day of ovum pick-up to transfer day in both groups. Serum PP14 in the pregnant group was significantly reduced (p<0,05) on pick-up day (0,91±0,78 ng/ml vs 2,39±2,28 ng/ml). Finally, serum LIF was significantly elevated (p<0,02) on pick-up day in the pregnant group (22,24±11,2 pg/ml vs 13,7±9,7 pg/ml). Conclusions: In conceptional cycles, during preimplantation stage, low PP14 and high LIF levels are the expression of enhanced immunosuppression and chemotaxis in the endometrium, making it more receptive to implantation.
Key words: *IVF-ET; LIF; PP14; VEGF; endometrial function; uterine artery Doppler velocimetry; ART*

INTRODUZIONE

Endometrial receptivity is affected by several endocrine and paracrine factors secreted by follicles, by the endometrial surface epithelium and stroma and by embryonic cells (1).
The successful outcome of an assisted reproduction technology (ART) cycle depends on a number of factors, including preparation of the endometrium in the embryo preimplantation stage, the quality of gametes and of the embryo, and the implantation and function of the placenta (2).
Implantation rates remain low despite the transfer of apparently healthy embryos, indicating that problems are encountered in the endometrium preparation steps, which are controlled by endocrine and paracrine factors (3, 4).
Embryo-endometrium interactions are characterized by complex molecular dynamics involving a variety of cytokines and growth factors – leukemia inhibitory factor (LIF) (5,6,7), interleukin 1 (IL1) (8), colony stimulating factor 1 (CSF1) (9), insulin-like growth factor binding protein 1 (IGFB1) (10), keratinocyte growth factor (KGF) (11), heparin binding epidermal growth factor (HB-EGF) (12), placental protein 14 (PP14) (13-14), cancer antigen 125 (CA125) (15-16),

vascular endothelial growth factor (VEGF) (17) – as well as hormonal factors such as P and inhibin A, and cell adhesion molecules such as mucin 1 (MUC1) (18) and integrins, which can be all be considered biochemical markers of endometrial receptivity. The results of studies on so-called biochemical markers of endometrial receptivity are conflicting and there is a lack of data on preimplantation stage in the literature, except for the reports of Westergraad (19) on natural reproduction cycles and of Chryssikopoulos (16) and Westergraad (62) on ART cycles.
Our study was aimed at investigating endometrial function by testing some markers, easily measurable in maternal serum, namely CA125, PP14, LIF, E_2 and P as well as the angiogenic cytokine VEGF (20) both in maternal serum and in a pool of follicular fluid collected during pick up from all follicles aspirated , and by studying uterine, radial and follicular arterial blood flow.
The primary end-point was to determine whether there were any significant endometrial function changes in the early luteal phase prior to embryo implantation in a sample of infertile women undergoing ART with respect to reproductive outcome (pregnancy vs non pregnancy).

METHODS

Case Series
We selected 35 women from 60 infertile couples referred for IVF-ET to the Center for medically assisted reproduction at the Department of Obstetrics & Gynecology and Neonatology of the Parma University Hospital between november 2003 and March 2004 (before the new Italian law 40/2004 on ART). Inclusion criteria were normoresponder women with menstrual cycles of 26 to 32 days, age under 40, and baseline FSH levels lower than 10 IU/L. Women with endocrine abnormalities, ovarian hyperstimulation syndrome, disorders of the uterus (e.g. uterine malformations or fibroids) or of the adnexa (e.g. hydrosalpinx, endometriosis or polycystic ovary) chemical or clinical abortion and ectopic pregnancies were excluded from the study. All 35 couples included in the study underwent ART cycles for moderate to severe male infertility.
Prior to study enter, all women signed their informed consent and were verbally informed about the methods and goals of the study. The study was approved by the University of Parma Bioethics Committee.
The selected women were divided into two groups depending on ART outcome: a group of 12 pregnant women and a group of 23 not-pregnant women.
Pregnancy was considered ongoing if hCG concentrations were ≥120 IU/L at day 16 after ovum pick-up and if transvaginal sonography showed the presence of an intrauterine gestational sac at day 35.

Stimulation Protocol
The women under study received a cycle of ovarian stimulation, beginning with pituitary desensitization by GnRH agonist leuprolide at a dose of 0.5 mg/day SC (Enantone die; Takeda Italia Farmaceutici, Rome, Italy) administered in the mid-luteal phase of the previous menstrual cycle.
Following ovarian suppression, follicular development was stimulated by recombinant FSH (rFSH) (Puregon, Organon Italia, Rome, Italy) at a standard dose of 225 IU, later adjusted according to each woman's response.
Follicular growth was monitored by serum E_2 assay and transvaginal sonography every other day. Ovulation was induced by administration of 10,000 IU of hCG (Gonasi, Amsa, Rome, Italy) if there was ultrasound evidence of at least two follicles of ≥16 mm in diameter and if serum E_2 levels were > 1,000 pg/mL.
Transvaginal ultrasound-guided ovum pick-up was performed under general anesthesia 35 hours after hCG administration. The retrieved mature oocytes were fertilized by intracytoplasmic sperm injection (ICSI).
The embryos thus obtained were classified according to the criteria proposed by Staessen (21). Embryo transfer was performed 3 days after ovum pick-up.
The luteal phase was supported by 90 mg of 8% P gel (Crinone 8, Serono Italia, Rome, Italy) administered vaginally from pick-up day to beta-hCG testing at day 16. If pregnancy was confirmed, the treatment was continued up to the 12th week of gestation.

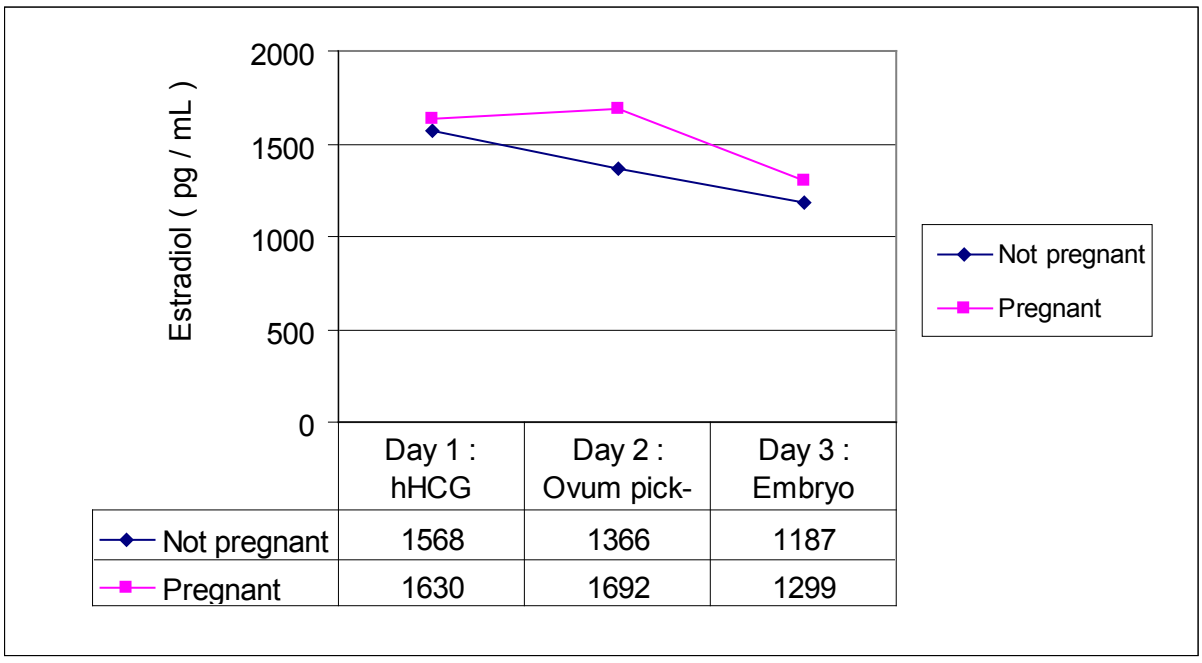
Hormone, Endometrial Protein Marker and Angiogenic Cytokine Tests
The women's blood serum samples were frozen at -70 °C for determination of the following endocrine and paracrine parameters: LH, on day 6 of stimulation; E_2, on hCG day, on the morning of pick-up day and on transfer day; serum PP14, P, LIF, and VEGF on

the morning of pick-up day and on transfer day; and, CA125, only on pick-up day, because this step eventually affects the accuracy of testing on transfer day.
Serum E_2, P and CA125 concentrations were determined in duplicate using an Immulite chemoluminescence kit (Diagnostic Product Corporation, Los Angeles, CA, USA; Italian distributor: Medical System., Genoa).
LH levels were tested by the Coat-A-Count LH IRMA Kit (Euro/Diagnostic Products Corporation, Witney, Oxfordshire, UK).
PP14 concentrations were assayed by glycodelin ELISA (Bioserv Diagnostics, Rostock, Germany; Italian distributor: AB Analitica, Padua).
LIF levels were tested by the Human ELISA Kit (Bender Med Systems Inc., Vienna, Austria; Italian distributor: Tebu-bio, Magenta).
Serum and follicular VEGF concentrations were determined using a Human VEGF ELISA Kit (R&D Systems; Minneapolis, MN, USA; Italian distributor: Space Import Export, Milan).
Intra- and interassay parameter variations were 4% and 4.2% for E_2, 2.2% and 4% for FSH, 1% and 2.2% for LH, 6.2 % and 6.7 % for P, 8,3.% and 4,3% for PP14, 5.5 % and 7.0% for LIF, 4,0% and .6,7% for CA125, respectively, and < 8% for VEGF.
Determinations were performed in duplicate both in serum and in the follicular fluid.
The follicular fluid pool from each patient was centrifuged at 900 rpm for 15 minutes to remove cellular and blood contamination and then frozen at -70° C.
Permission for use of the follicular fluid and blood samples for this study was given by the University of Parma Ethics Committee. All patients gave their informed consent to IVF-ET.
Transvaginal Ultrasound Assessment
Transvaginal sonography was performed using an AU5 Harmonic ultrasound system with a 5-7.5Hz transvaginal convex transducer (Esaote Biomedica, Genoa, Italy), equipped with a pulse wave Doppler system for quantitative blood flow measurement.
On the day of hCG administration, all women had a transvaginal ultrasound examination to evaluate endometrial thickness measured at the maximum thickness between the interfaces of the endometrial-myometrial junction through the longitudinal axis of the uterus after obtaining a proper longitudinal view of the uterus. A second transvaginal ultrasound examination was performed on pick-up day to evaluate uterine, endometrial and follicular vascularization by color Doppler flow velocity measurement of the uterine, radial and follicular arteries according to Kurjac's technique (22). The measured flow velocimetry parameters were resistance index (RI), pulsatility index (PI), and peak systolic velocity (PSV) : RI, calculated with the formula RI = (S/D)/S, where S is peak systolic velocity and D is end-diastolic flow velocity; PI, calculated with the formula PI = (S-D)/M, where M is the time-averaged maximum velocity, and PSV expressed in cm/s.
Uterine perfusion was measured at the uterine arteries, viewed laterally to the cervix in a longitudinal scan at the level of the uterine cervix-isthmus junction. Endometrial vascularization was measured at the radial arteries. Ovarian blood flow was assessed by measuring perifollicular vascularization at the dominant follicle (≥16 mm in diameter).
All ultrasound examinations were performed by the same sonographer between 7:30 and 9:00 a.m., within 60 minutes of ovum pick-up and with the women in the lithotomy position.

Statistical Analysis
Data were expressed as means ± SD. Statistical analysis was performed using Excel Office 2003 for Student's test. A *P* value of < 0.05 was considered statistically significant.

RESULTS
Comparative data analysis showed that the two study groups were homogeneous, with no significant differences in age, baseline FSH levels, administered total dosage of rFSH, endogenous LH levels at day 6 of the stimulation cycle, endometrial thickness on hCG day, and E_2 levels on hCG (Table 1), pick-up (Table 1) and transfer (Table 1, Fig. 1) days. However, there was a significant (P = 0.04) decline in E_2 concentrations from hCG day to transfer day in the not-pregnant group (1,568 ± 552 pg/mL and 1,187 ± 652 pg/mL, respectively) versus the pregnant group (1,630 ± 1,043 pg/mL and 1,299 ± 800 pg/mL, respectively; P = not significant) (Fig. 1).
No statistically significant differences were observed between the two study groups in the following parameters: number of retrieved oocytes, number and type of embryos obtained, and number of embryos transferred into the uterus (Table 2); serum CA125

Figure 1. Trend of mean E_2 values from hCG day to embryo transfer day.



Pregnant: 1 vs 3, not significant; Not pregnant: 1 vs 3, $P = 0.04$

Table 1. Comparative analysis of baseline clinical data during controlled ovarian hyperstimulation and of endometrial thickness on the day of hCG administration

	Pregnant	Not pregnant	*P*
No. of cases	12	23	
Age	33.3 ± 3.74	33.4 ± 2.6	NS
Baseline FSH (IU/L)	7.47 ± 2,2	6.79 ± 1.68	NS
LH at day 6 (IU/L)	0.9 ± 0.68	1.44 ± 1.1	NS
E_2 on hCG day (pg/mL)	1,630.6 ± 1,043.6	1,503.7 ± 580.7	NS
rFSH (IU/patient)	3,018 ± 720	3,469.5 ± 1,340.3	NS
Endometrial thickness (mm) on hCG day	10.72 ± 3.1	10.03 ± 2.2	NS

Note: Values are means ± SD. NS = not significant.

concentrations on pick-up day (Table 3); serum VEGF (on pick-up day and transfer day) and follicular VEGF (on pick-up day) concentrations (Table 4); and follicular and endometrial blood flow velocity, assessed by measuring the RI, PI and PSV of the uterine, radial and follicular arteries on pick-up day. (Table 4).

Serum P levels rose significantly from pick-up day to transfer day both in the pregnant group (19.63 ± 9.72 ng/mL vs 104.5 ± 33.1 ng/mL; $P < 0.02$) and in the not-pregnant group (14.63 ± 5.75 ng/mL vs 90.8 ± 28,36 ng/mL; $P < 0.05$). Serum PP14 levels were also increased in pregnant (0.91 ± 0,78 ng/mL vs 4.66 ± 2.91 ng /mL, $p < 0.0002$) and in not-pregnant women (2.39 ± 2.28 ng/mL vs 7.69 ± 5.24 ng/mL, $p < 0.001$) (Table 3).

However, PP14 levels in the pregnant group were significantly reduced ($P < 0.05$) compared with the not-pregnant group on pick-up day (0.91 ± 0.78 ng/mL vs 2.39 ± 2.28 ng/mL, $P < 0.05$) (Table 3) .

Serum LIF levels were significantly elevated ($P < 0.02$) on pick-up day in the pregnant versus the not-

Table 2. IVF data.

	Pregnant	Not pregnant	*P*
No. of cases	12	23	
Retrieved oocytes (total)	8.75 ± 4.35	8.78 ± 3.86	NS
Embryos obtained (total)	4.08 ± 1.78	3.95 ± 1.87	NS
No. of type-1 embryos	2.83 ± 1.94	2.26 ± 1.78	NS
No. of type-2 embryos	3 ± 2.04	3.39 ± 1.99	NS
No. of transferred embryos	3.08 ± 0.78	2.91 ± 1.08	NS
Fertilization rate (%)	50.8 ± 16.3	47.7 ± 17.8	NS

Note: Values are means ± SD. NS = not significant.

Table 3. Endometrial and corpus luteum function data.

	Pregnant	Not pregnant	*P*
No. of cases	12	23	
Serum P on ovum pick-up day (ng/mL)	19.63 ± 9.72^{a}	14.63 + ± 5.75$^{a\text{-}1}$	NS
Serum P on embryo transfer day (ng/mL)	104.5 ± 33.1^{b}	90.8 ± 28.36$^{b\text{-}1}$	NS
Serum PP14 on ovum pick-up day (ng/mL)	0.91 ± 0.78^{c}	2.39 ± 2.28$^{c\text{-}1}$	< 0.05
Serum PP14 on embryo transfer day (ng/mL)	4.66 ± 2.91^{d}	7.69 ± 5.24$^{d\text{-}1}$	NS
Serum CA125 on ovum pick-up day (IU/mL)	9.23 ± 3.78	9.72 ± 3.71	NS
Serum LIF on pick-up day (pg/mL)	22.24 ± 11.2^{e}	13.7 ±9.7$^{e\text{-}1}$	< 0.02
Serum LIF on embryo transfer day (pg/mL)	22.61 ± 24.28^{f}	17.09 ± 11.5$^{f\text{-}1}$	NS
Serum E_2 on hCG day	1.630 ± 1.043^{g}	1,568 ± 552$^{g\text{-}1}$	NS
Serum E_2 on ovum pick-up day	1,692 ± 1,582^{b}	1,366 ± 607$^{b\text{-}1}$	NS
Serum E_2 on embryo transfer day	1,299 ± 800^{i}	1,187 ± 653$^{i\text{-}1}$	NS

Note: Values are means ± SD. NS = not significant.
^{a} vs ^{b}: p < 0.02; $^{a\text{-}1}$ vs $^{b\text{-}1}$: p < 0.05; ^{c} vs ^{d}: p < 0.0002; $^{c\text{-}1}$ vs $^{d\text{-}1}$: p < 0.001; ^{e} vs ^{f}: NS; $^{e\text{-}1}$ vs $^{f\text{-}1}$: NS; $^{g\text{-}1}$ vs $^{i\text{-}1}$: p = 0.04; ^{e} vs ^{f}: NS; ^{g} vs ^{i}: NS.

pregnant group (22.24 ± 11.2 pg/mL vs 13.7 ± 9.7 pg/mL (Table 3).

DISCUSSION

The study of endometrial receptivity at embryo transfer (23) and the identification of endometrial function markers (2) is crucial not only to an understanding of the physiological mechanisms of embryo implantation, but also to its use in clinical practice.

In this study, we investigated changes in serum and follicular concentrations of some endocrine and paracrine markers that can be measured in maternal serum during ART cycles, namely the P-associated cytokines CA125, PP14 and LIF, the angiogenic cytokine VEGF, E_2, related to folliculogenesis and endometrial proliferation, and P, related to luteal function and endometrial secretion.

E_2 concentrations on hCG, pick-up and transfer days did not show statistically significant differences between the pregnant and not-pregnant groups. How-

Table 4. VEGF and blood flow velocity indices.

	Pregnant	Not pregnant	*P*
No. of cases	12	23	
Serum VEGF on ovum pick-up day (pg/mL)	192.55 ± 100.81	238 ± 169	NS
Serum VEGF on embryo transfer day (pg/mL)	199.7 ± 128.78	272 ± 259	NS
Follicular VEGF on ovum pick-up day (pg/mL)	4,277 ± 1,270	3,440 ± 1,341.28	NS
UTERINE ARTERIES (middle)			
RI	0.77 ± 0.07	0.79 ± 0.06	NS
PI	1.92 ± 0.46	1.95 ± 0.37	NS
PSV (cm/s)	33.7 ± 16.9	33.72 ± 9.9	NS
RADIAL ARTERY			
RI	0.83 ± 0.13	1.01 ± 0.05	NS
PI	2.46 ± 1.42	2.01 ± 0.89	NS
PSV	8.55 ± 2.42	8.98 ± 5.08	NS
FOLLICULAR ARTERY			
RI	0.5 ± 0.07	0.46 ± 0.06	NS
PI	0.75 ± 0.15	0.71 ± 0.16	NS
PSV (cm/s)	14.8 ± 3.89	13.41 ± 3.46	NS

Note: Values are means ± SD. NS = not significant.

ever, E_2 levels appeared to decrease significantly from hCG day to transfer day (Fig. 1) only in the non pregnant group. The role of oestradiol in the lutheal phase is unclear and the definition of optimal oestrogen conditions for implantation could be useful . Although Hung Yu Ng *et al* (24) did not find an adverse outcome in IVF cycles with rapid decline in serum oestradiol concentration around the mid-luteal phase , our data are in agreement with those of Sharara *et al* (25) and Gruber *et al* (26). Sharara *et al* (25) demonstrate that the ratio of day of HCG oestradiol to mid-luteal oestradiol was highly predictive of successful outcome in IVF-embryo transfer : the ongoing pregnancy rate and implantation rate were significantly impaired if the above ratio exceeded 5.0 . Gruber I et al. (26) demonstrate that the high E2/P ratio at the time of embryo transfer is significantly higher in conceptional vs not conceptional cycles and preclinical abortions (18,1 +/-11.3 vs 8,7 +/-7,9 and 9,3 +/-6,3 , P < 0,005).

Serum P levels showed a significantly positive trend from pick-up day to transfer day, with no differences between the two study groups. This demonstrates that luteal function was adequate in both groups, but luteal E_2 played a permissive role only on the endometrium of women who conceive, given the slow decrease of E_2 from hCG day to transfer day in pregnant women (*P* not significant).

Angiogenic function, which is fundamental for embryo implantation and development (17), did not show any statistically significant differences in the two study groups when measured by both serum and

follicular VEGF assay. The correlation of serum and follicular VEGF concentrations to successful outcome of ART cycles with respect to endometrial and follicular vascularization is still controversial (27, 28).
A few authors believe that elevated follicular VEGF concentrations indicate a rich vascularization and abundant oxygenation of ovarian follicles, resulting in better quality of fertilized embryos (29).
On the other hand, others claim that elevated follicular VEGF concentrations are the result of hypoxic stimulation within the follicle (20, 30, 31).
In our study, the vascular factor was investigated also by flow velocity measurement in uterine, endometrial and follicular perfusion, in order to detect any possible correlation between adequate blood supply to the uterus and the ovaries, embryo quality, and endometrial receptivity (32, 33)
The two study groups were homogenous before embryo transfer, with no statistically significant differences not only in follicular and uterine-ovarian vascularization, but also in endometrial thickness and embryo quality.
An evaluation of endometrial function expressed by the three endometrial cytokines (CA125, PP14 and LIF) did not reveal any significant differences in CA125 between the pregnant and not-pregnant groups, proving that this marker still has a controversial role as an indicator of endometrial function and a predictor of successful embryo implantation in ART (34, 35). Such evidence is in contrast to that reported by Tavmergen *et al* (36), who showed higher CA125 levels in pregnant women than in not-pregnant women on the day of ovulation trigger during ART cycles. In their study, Chryssikopoulos *et a.* (16), found comparable serum concentrations on trigger day, but significantly higher concentrations on pick-up day in pregnant women – a difference that was not observed on transfer day. In contrast to previous studies, Noci *et al* (37) found significantly lower CA125 levels in pregnant women than in non-pregnant women on pick-up day. Dalton *et al* (38) did not find any differences in CA125 concentrations in uterine secretions during the luteal phase in a group of women with recurrent abortion compared with a group of fertile controls.
In both our study groups, circulating PP14 levels rose from pick-up day to transfer day, confirming the findings of Julkunen *et al* (14), Chryssikopoulos A (16) ,Westergaard (19) , Andersen (60) and Westergaard (62) who reported increased concentrations of this endometrial protein in the mid-luteal phase and in early gestation, probably as a result of increasing P levels. PP14 is a glycoprotein synthesized by the secretory endometrium and decidua and has been proposed as a biochemical marker for the evaluation of endometrial function (12, 39, 40).
Of particular importance is the local immunomodulating action exerted by this protein, which favors embryo implantation by regulating the immune response of the maternal endometrium to paternal/embryonic antigens (41).
PP14 directly inhibits the proliferation of T-cells and the secretion of T-helper-1 (Th1)-dependent cytokines, especially interleukin 2 (IL2), which are responsible for the cell-mediated immune response to embryonic antigens (42, 43, 44)
The immunoinhibitory action on Th1 cells favors the Th1/Th2 shift, resulting in an increased concentration of Th2-dependent cytokines, such as interleukin 4 (IL4) and LIF, that favor the maternal/embryonic immune interaction (44, 45, 46, 47, 48, 49, 50, 51).
In addition, PP14 inhibits the cytotoxic activity of uterine natural killer cells and the NK inhibition was dependent on concentrations of PP14 (52).
As it does not act on Th2 cells, PP14 is thought to favor production of cytokines that are fundamental to maintain pregnancy, such as LIF (42, 44 ,46, 47, 53, 54). LIF is a multifunctional glycoprotein belonging to the interleukin 6 (IL6) family. Maximal expression is observed between days 19 and 25 of the menstrual cycle, coinciding with the time of human implantation (5) . During pregnancy, LIF genes and LIF receptor genes have been detected in the decidua and chorionic villi in the first trimester and in the placenta at term (55, 56 ,57). P seems to be the major regulator of LIF expression. It has been observed that treating women with P-receptor antagonists, such as mifepristone (RU-38486, Roussel Uclaf, Romainville, France), soon after ovulation reduces immunoreactivity to LIF at the time of implantation (58). A very important role played by LIF is to act as a chemotactic factor on natural killer cells and macrophages, controlling the migration of the cells to the site of implantation (59).
Local immunosuppressive activity exerted by PP14 is therefore of fundamental importance in regulating the immune mechanisms of embryo implantation. If we compare PP14 levels in the pregnant group with

those in the not-pregnant group, we can see that serum concentrations are significantly reduced ($P < 0.05$) in pregnant versus not-pregnant women, both individually on pick-up day .

This lends support to the hypotheses advanced by Westergaard (19-62) and Andersen (60) who assumed an increased uptake of thus glycoprotein by the endometrium in the preimplantation stage , because it is needed to suppress the maternal immune response. In addition, the significantly ($P < 0.02$) elevated LIF levels (Table 3) on pick-up day in women who conceived clearly suggest a favoring effect of this cytokine on pregnancy maintenance (5,42, 44, 46, 53, 54). Westergaard (19- 62) and Andersen (60) found low concentration of PP 14 in women who conceive in the late lutheal phase of cycle antecedent to and during ovarian stimulation but non in pick up day Tulppala *et al* (61) found low levels in the late luteal phase of this glycoprotein in infertile women or in women with recurrent abortion. On the other hand, in the study conducted by Seppälä *et al* (39) , plasma PP14 concentrations did not show significant differences between pregnant and not-pregnant women during ART cycles, while a difference was found after implantation.

Our study is unique in the literature, given the homogenous characteristics of the two study groups with respect to embryo quality, perifollicular vascularization, uterine perfusion, and endometrial thickness as well as luteal function. In addition the results are difficult comparable with another mentioned study but , even from our data, we could confirm the hypotheses of increased uptake of PP 14 by the endometrium in the preimplantation stage of conceptional cycles. In conclusion the only significant differences that we found in women who conceived versus women who did not, were the slow decrease of luteal E_2 from hCG day to transfer day, a reduction in PP14 levels, and an increase in LIF levels. While E_2 plays a permissive role in endometrium maturity of the early luteal phase , the decreased PP14 and the increased LIF levels are the expression of an immunosuppressive and a chemotactic action, respectively, that prepare the endometrium for optimal embryo implantation. Clinically, the evaluation of endometrial function is perhaps a step that should not be neglected when drawing a balance of the factors involved in successful ART cycles. While further studies are needed to evaluate their predictivity, sensitivity and specificity and to predict which women will have a clinical pregnancy , PP14 and LIF are two glycoproteins that play a fundamental role in the preimplantation stage.

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