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# LIF endometrial expression is impaired in women with unexplained infertility while LIF-R expression in all infertility sub-groups

Chrysoula Margioula-Siarkou[a,b,*], Yannis Prapas[a,b,1], Stamatios Petousis[a,b,1], Stefanos Milias[c], Konstantinos Ravanos[b], Themistoklis Dagklis[a], Ioannis Kalogiannidis[a,b], George Mavromatidis[b], Constantinos Haitoglou[d], Nikolaos Prapas[a,b,1], David Rousso[a,b,1]

[a] 3rd Department of Obstetrics and Gynaecology, Aristotle University of Thessaloniki, Greece
[b] IAKENTRO, Infertility Center, Thessaloniki, Greece
[c] Pathology Division, 424 General Army Hospital, Thessaloniki, Greece
[d] Laboratory of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, Greece

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ABSTRACT

The main objective of our study was to study LIF and LIF-R endometrial expression during the implantation window in the various sub-groups of infertile women according to infertility cause. A prospective observational case-control study was performed from March 2013 to February 2016. Infertile women consisted of the patients' group (group 2) while fertile women were the control group (group 1). Infertile women were divided according to infertility cause in women with tubal factor (group 2a), poor ovarian reserve (group 2b), endometriosis (group 2c) and unexplained infertility (group 2d). Endometrial biopsy was performed on 7th–8th postovulatory menstrual day. Leukemia Inhibitory Factor (LIF) and LIF-Receptor (LIF-R) expression in epithelial and stromal cells were assessed with Immunohistochemistry (IHC). There were 20 infertile with poor ovarian reserve, 15 with tubal factor, 10 with endometriosis and 15 with unexplained infertility included in the analysis. LIF expression in patients with unexplained infertility was significantly compared with controls ($P = 0.006$). No significant difference was observed between patients with tubal factor, poor ovarian reserve and endometriosis compared with control group ($P = 0.78$, $P = 0.44$ and $P = 0.56$ respectively). Analysis of LIF-R expression in sub-categories of infertility indicated that expression was significantly decreased in all sub-groups of infertility. Our study indicated impaired LIF expression levels only in women with unexplained infertility, while LIF-R expression was impaired in all sub-groups of infertile women. Further multicenter prospective studies should be performed in order to assess the exact etiopathogenetic role of these cytokines in the molecular background of infertility.

## 1. Introduction

Success of implantation requires both appropriate embryo development to the blastocyst stage and differentiation of the endometrium to a receptive state after proper morphological and cellular modifications [1]. The endometrium is receptive during a specific interval which occurs in the mid-luteal phase of the menstrual cycle and is called the "implantation window" [2]. Apposition, adhesion and invasion of the blastocyst requires a dialogue between the blastocyst and the endometrium, which is mediated by several hormones, cytokines, enzymes and growth factors [3]. These mediators of implantation should be expressed during this critical interval both in the embryo and the endometrium, so that implantation is achieved and further normal development of a viable pregnancy is assured (see Images 1–4).

Leukemia Inhibitory Factor (LIF) is a pleiotropic-secreted cytokine of the IL-6 family that may act on various tissues and cell types [4,5]. LIF actions are mainly induced after binding to the LIF cell-surface receptor (LIF-R), which is an heterodimer consisting of two sub-units, the gp130 receptor and the LIF-R alpha (LIF-Ra) sub-unit. LIF-Ra selectively interacts with LIF, while gp130 may also interact with other cytokines. LIF is initially connected to LIF-Ra with low-affinity binding, which in turn induces the dimerization with gp130 leading to a high-affinity receptor [2,5–7]. Development of the heterodimer receptor motivates multiple intracellular signaling pathways such as PI3K (Posphatidylinositol-3-Kinase Pathway), MAPK (Mitogen-Activated Protein Kinase) and JAK/STAT (Janus Kinase/Signal Transducer and Activator

* Corresponding author at: Konstantinoupoleos 49 str, 56242 Thessaloniki, Greece.
*E-mail address:* m.s.chrysoula@mail.gr (C. Margioula-Siarkou).
[1] To be mentioned as equal authors.

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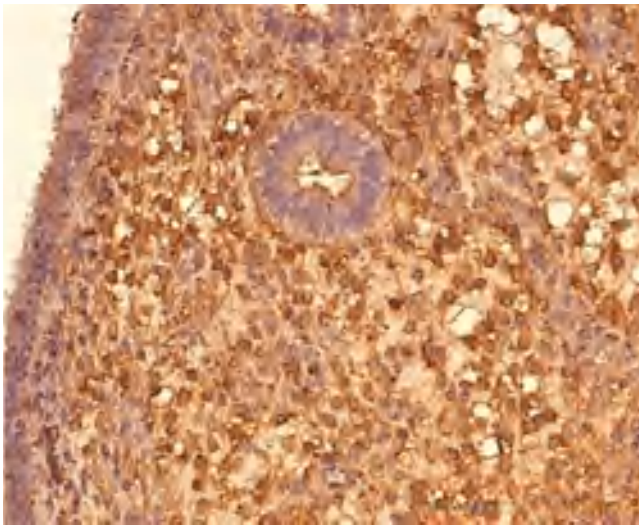

**Image 1.** LIF expression in endometrial cells of fertile patient.

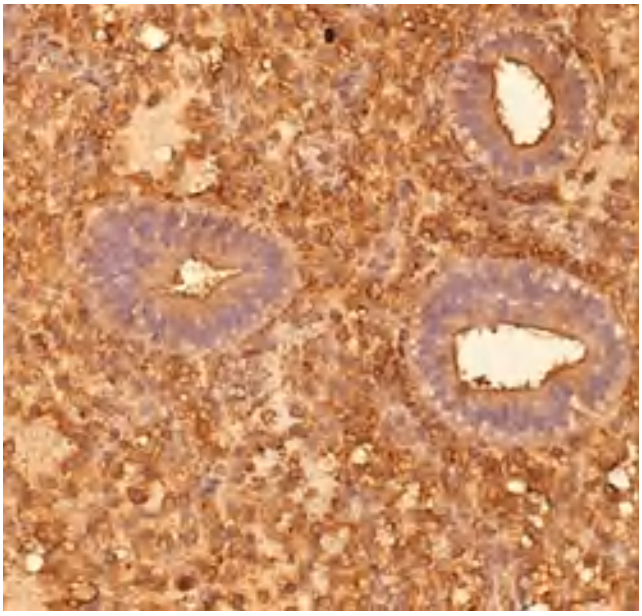

**Image 2.** LIF-R expression in endometrial cells of fertile patient.

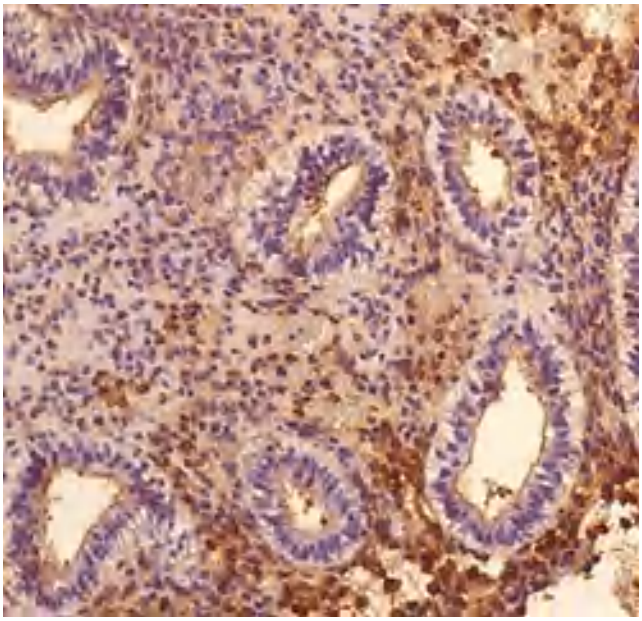

**Image 3.** LIF expression in endometrial cells of patient with unexplained infertility.

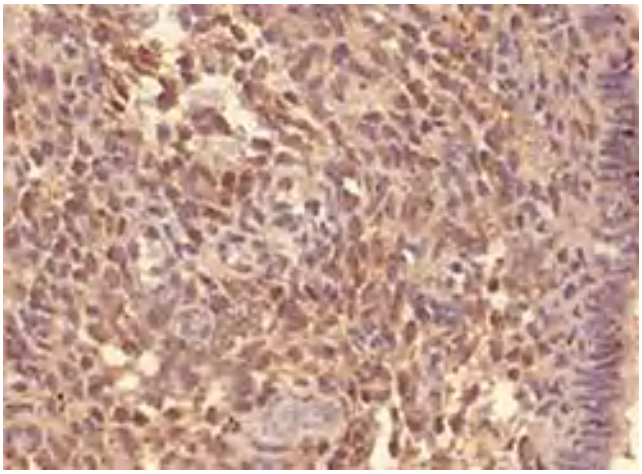

**Image 4.** LIF-R expression in endometrial cells of patient with endometriosis.

of Transcription), through which LIF performs its multiple actions [1]. Specifically, LIF contributes to both the adhesion and invasion stages of implantation, and also participates in the modulation of trophoblast invasiveness by controlling HLA-G expression of invasive cyto-trophoblast cells [8,9].

LIF, and consequently LIF-R involvement in the implantation procedure, requires appropriate expression levels during the implantation window. LIF and LIF-R expression levels gradually increase after ovulation, which continues until the end of the menstrual cycle. It has been reported that LIF concentration is maximized between the 7th and 12th postovulatory day, while the levels of LIF-R and gp130 reach a peak between the 19th and 25th day of the cycle [10–12]. It has also been observed that stronger LIF immunoreactivity during the implantation window is correlated with a higher possibility of pregnancy, while decreased LIF expression during this interval is associated with a lower possibility of conception in subsequent cycles [13]. Thus, maximization of LIF and consequently LIF-R levels is essential so that these cytokines can contribute towards implantation.

Pilot results of our work as well as other published studies rather conclude that LIF expression is decreased in the general population of infertile women during implantation window [14–18]. However, the main clinical interest would be to specify in which sub-groups of infertile women, according to cause of infertility, levels are impaired. Furthermore, most studies have focused on gp130 rather than LIF-R, despite the fact that LIF-Ra selectively interacts with LIF. As a result, there is no published study examining LIF and LIF-R expression patterns in the various sub-groups of infertile women during implantation window.

The main objective of our study was to assess LIF and LIF-R epithelial and stromal cell expression in various sub-groups of infertile women based on the cause of infertility.

## 2. Materials and methods

A prospective observational case-control study was performed from March 2013 to February 2016. Women who had failed to conceive after at least one year of contraceptive-free sexual intercourse comprised the patients' group (group 2) [19], while women having delivered at least one live newborn without signs or symptoms of infertility afterwards were the control group (group 1). Detailed medical histories were obtained to divide the infertile women into 4 main sub-groups, according to the cause of infertility: women with tubal factor infertility

(group 2a), poor ovarian reserve (group 2b), endometriosis (group 2c) and unexplained infertility (group 2d). All women included in the study were aged less than 42 years. Any history of gynecological malignancy, polyps, hyperplasia or recent surgical procedures was predefined as an exclusion criterion. Fertile women with a history of miscarriage or ectopic pregnancy were also excluded. Informed consent was obtained from all women participating in the study. The Institutional Review Board and the Ethical Committee of Aristotle University of Thessaloniki approved the present study.

### 2.1. Description of intervention

Each woman's ovulation day was determined using serial ultrasound scans and LH measurement. Vaginal ultrasound was performed on the 2nd menstrual day to rule out the presence of ovarian cysts. Menstruation was spontaneous in all women without usage of contraceptive pills. Transvaginal ultrasound scan was performed daily beginning on the 8th menstrual day. The maximum diameter of the predominant follicle was measured. The menstrual day on which maximum follicle diameter was observed, followed by elimination or heterogeneity of clear ultrasound limits on the next menstrual day, was considered as the ovulation day. Cycles in which no follicle with a mean diameter over 18 mm was observed were considered as unovulatory and were excluded from the analysis.

Endometrial biopsy was performed with a Pipelle de Cornier® on the 7th–8th postovulatory menstrual day. All biopsies were performed by the same physician (Y.P.) under abdominal ultrasound guidance. The collected tissue was added to a 10% formalin solution and was sent for Immunohistochemistry (IHC) analysis. IHC was performed by a specialized pathologist that was unaware of the sample origin (fertile/infertile) and the menstrual day of the biopsy (blind examiner).

### 2.2. Immunohistochemistry (IHC)

IHC procedure has been described elsewhere [14]. Each specimen was fixed in 10% buffered formalin solution for twelve hours. All specimens underwent overnight dehydration in an automated closed type tissue processor as well as paraffin embedding. Sections of 3.5 μm were cut from each paraffin block with a rotary microtome and were set in positively charged superfrost microscopic slides. These slides were used for immunohistochemical stains, while hematoxylin and eosin staining of another plain microscopic slide was also performed. The positively charged slides were deparaffinized in an incubator at 64.5 °C for 45 min. Immunostaining was performed with the use of an automated immunostainer (Bond, Leica Microsystems). The immunostainer uses a kit for the detection of primary antibodies (Bond Polymer Refine Detection, Leica Biosystems Ltd, Newcastle, United Kingdom) which contains 3.0% hydrogen peroxide, a polymer penetration enhancer (post primary), polymer-HRP anti mouse/rabbit IgG, DAB (3,3′-Diaminobenzidine tetrahydrochloride) and hematoxylin.

Polymer Refine Detection utilizes a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. The detection system avoids the use of streptavidin and biotin, and therefore eliminates non-specific staining as a result of endogenous biotin. The specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity. Post Primary IgG linker reagent localizes mouse antibodies. • Poly-HRP IgG reagent localizes rabbit antibodies. The substrate chromogen, 3,3′-Diaminobenzidine tetrahydrochloride hydrate (DAB), visualizes the complex via a brown precipitate. Hematoxylin (blue) counterstaining allows the visualization of cell nuclei.

All reagents should be equilibrated to room temperature (20–25 °C) prior to immunostaining. Likewise, all incubations should be performed at room temperature. Tissue sections mounted on glass slides positively charged deparaffinized with DEWAX solution ready to use at the immunostainer and then rehydrate with wash buffer dilution. After deparaffinization and hydration to buffer (water), the tissue sections should be subjected to heat induced epitope retrieval (HIER), at 98 °C at the immunostainer with citrate buffer pH 6 (ER1) for 20 min.

The antibody used for LIF detection was rabbit polyclonal, concentrate 100 ug/ml, at a final dilution 1:200 (Atlas/Sigma U.S.A., code: HPA018844) and for LIF-R rabbit polyclonal, concentrate 200 ug/ml, at a final dilution 1:100 (C-19, Santa Cruz, U.S.A., code: sc-659).

Each staining run included a known positive control specimen (kidney and lung tissue) to ascertain a proper performance of all the applied reagents. If the positive control specimen fails to show positive staining, labeling of test specimens should be considered invalid. A negative control reagent used with each specimen to identify non-specific staining. If non-specific staining cannot be clearly differentiated from the specific staining, the labeling of the test specimen should be considered invalid.

The diaminobenzidine-containing Substrate Working Solution gives a brown color at the site of the target antigen recognized by the primary antibody. The brown color should be present on the positive control specimen at the expected localization of the target antigen. If non-specific staining is present, this will be recognized as a rather diffuse, brown staining on the slides treated with the negative control reagent. Nuclei will be stained blue by the hematoxylin counterstain.

Histological dating of the endometrium was assessed according to the histological criteria described by Noyes et al. [20] A difference of over 3 days between histological and chronological dating characterized the sample as out-of-phase. IHC staining was assessed by optical microscopy. Liver, kidney and lung tissues were used as control samples. An endometrial sample was considered positive when the cell was stained brown. The percentage of positive cell staining was measured in every sample. Staining intensity was quantified on a 0–3 subjective score scale where 0 corresponds to no staining, 1 to mild staining, 2 to moderate staining and 3 to intense staining. Staining was scored in accordance to the full intensity staining observed in control tissues. The H-score was defined as $\Sigma xi(i + 1)$ of positive cell percentage and staining intensity ranging from 0 to 300 [21]. Indeed, the h-score was assessed as the summary of as the mathematical product of percentage of positive cell staining and intensity of staining. For example, in case the percentage was 70% and this was scored with 2 regarding intensity (moderate intensity), the final h-score would be 140. The former parameters were examined separately for epithelial and stromal cells. Scoring of all tissues was performed blindly by the same physician (S.M.)

### 2.3. Primary and secondary outcomes – epidemiological characteristics

The percentage of positive cellular staining, staining intensity and H-score of LIF and LIF-R expression in epithelial and stromal cells of fertile and infertile women were set as primary outcomes. Secondary outcomes were the endometrial dating of obtained samples as well as the rate of out-of-phase endometrial tissues in various groups.

Epidemiological characteristics, with special interest in the obstetrical history of fertile women and the infertility history of infertile women (including previous In Vitro Fertilization (IVF) attempts and their outcome), were recorded. The menstrual day on which the biopsy was performed, the interval from ovulation day to biopsy and endometrial thickness at biopsy were also examined.

### 2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 21.0 (SPSS 21.0, Chicago). The mean values, standard deviation and standard error of the mean were estimated for continuous variables, while categorical variables were expressed as percentages. The normality of numeric variables was tested using the Kolmogorov-Smirnov test. The independent samples *t*-test was used for the comparison of normally distributed variables and the Mann-Whitney test for

the non-normally distributed variables. Fisher's exact test (chi-square criterion) was used for categorical parameters. Linear regression was used to assess potential correlation for primary outcomes with age. Both primary and secondary outcomes were compared between each sub-group of infertility and control group. Statistical significance was defined at $P < 0.05$.

## 3. Results

There were overall 30 fertile and 75 infertile women meeting the inclusion criteria that were enrolled in the study. Ovulation was confirmed in 24 fertile and 65 infertile women. Adequate endometrial tissue was finally obtained in 20 fertile and 60 infertile women. There were 3 women in group 1 and 3 women in group 2 for whom the procedure was discontinued because of pain and 2 cases in both groups in which not enough tissue sample was obtained to perform proper measurements, according to the pathologist.

Mean age was 31.3 ± 4.3 for fertile vs. 37.4 ± 4.1 for infertile patients ($P < 0.001$). No significant difference was observed regarding gynecological history parameters. Regarding the cause of infertility, there were 20 infertile women with poor ovarian reserve, 15 with tubal factor infertility, 10 with endometriosis and 15 with unexplained infertility. The mean interval from infertility diagnosis was 4.7 years, while a mean number of 2.1 ART attempts per woman had already been performed before they were recruited to the present study. Epidemiological characteristics for both fertile and infertile women are shown in Table 1.

### 3.1. Primary outcomes

LIF epithelial expression was significantly decreased only in the sub-group of women with unexplained infertility compared with control group. H-score was 106.3 ± 19.6 for control group vs. 38.9 ± 9.9 for women with unexplained infertility ($P = 0.006$). No significant difference was observed between patients with tubal factor infertility, poor ovarian reserve and endometriosis compared with the control group ($P = 0.78$, $P = 0.44$ and $P = 0.56$ respectively). LIF expression in stromal cells was also comparable between controls and all infertility sub-categories.

Analysis of LIF-R expression in infertility sub-categories indicated that expression was significantly decreased in all infertility sub-groups. The H-score was 136.8 ± 14.9 for patients with tubal factor infertility ($P = 0.02$ vs. controls), 130.3 ± 9.1 for patients with poor ovarian reserve ($P = 0.02$ vs. controls), 91.0 ± 6.2 for patients with endometriosis ($P < 0.001$ vs. controls) and 134.2 ± 14.3 for patients with unexplained infertility ($P = 0.01$ vs. controls). Patients with endometriosis also presented significantly lower LIF-R expression in stromal cells compared with controls ($P = 0.008$). LIF and LIF-R expression in various sub-groups of infertile women is presented in Table 2. Boxplots for LIF and LIF-R expression of the various sub-groups are presented in Figs. 1 and 2.

As maternal age was significantly different between groups of the study, in order to exclude potential bias of age on primary outcomes, linear regression was examined between age and primary outcomes of study, namely LIF epithelial h-score, LIF stromal h-score, LIF-R epithelial h-score and LIF-R stromal h-score. No significant regression was observed between primary outcomes and age. Indeed, p values were 0.089 for LIF epithelial h-score, 0.37 for LIF-R epithelial h-score, 0.076 for LIF stromal h-score and 0.61 for LIF-R stromal h-score. Therefore, no significant regression was detected between age and primary outcomes, therefore excluding the potential of significant bias of age on primary outcomes.

### 3.2. Secondary outcomes

Biopsy-to-dating difference was significantly higher in patients with unexplained infertility, endometriosis and tubal factor compared with fertile controls ($P = 0.04$, $P = 0.04$ and $P = 0.05$ respectively). All sub-groups of infertility presented significantly increased rate of out-of-phase tissues compared with controls. Endometrial biopsy characteristics of sub-groups of infertile and controls are reported in Table 3.

**Table 1**
Epidemiological characteristics of patients included in the present study.

| Parameters | Fertile (group 1) n = 20 | Infertile (group 2) n = 60 | P value |
|---|---|---|---|
| *Personal characteristics* | | | |
| Age (years)[#] | 31.3 ± 4.3 | 37.4 ± 4.1 | < 0.001 |
| Height (cm)[#] | 1.68 ± 0.1 | 1.65 ± 0.07 | 0.32 |
| Weight (kg)[#] | 71.0 ± 11.2 | 68.7 ± 12.7 | 0.58 |
| *Gynaecological history* | | | |
| Menarche (years)[#] | 13.1 ± 1.1 | 13.0 ± 1.7 | 0.92 |
| Menstrual cycle (days)[#] | 28.2 ± 1.3 | 27.6 ± 1.4 | 0.41 |
| Menstruation (days)[#] | 4.3 ± 1.1 | 4.5 ± 0.9 | 0.68 |
| *Obstetrical history* | | | |
| Gravida[∞] | 2.6(1–5) | 0.6(0–3) | < 0.001 |
| Parity[∞] | 1.8(1–3) | – | |
| Abortion[∞] | 0.9(0–3) | | – |
| Miscarriage | – | −0.3(0–3) | |
| *Infertility history* | | | |
| Cause | | | |
| Poor ovarian reserve[†] | – | 20(33.3) | – |
| Tubal factor[†] | – | 15(25.0) | – |
| Endometriosis[†] | – | 10(16.7) | – |
| Unexplained infertility[†] | – | 15(25.0) | – |
| Interval from infertility diagnosis (years)[∞] | – | 4.7(1–14) | |
| Previous ART efforts[∞] | – | 2.1(0–16) | – |
| Previous IUI efforts[∞] | – | 0.6(0–4) | – |
| Previous IVF efforts[∞] | – | 1.6(0–15) | – |
| Previous Natural Cycle[∞] | – | 0.2(0–6) | – |
| Biochemical pregnancies[∞] | – | 0.1(0–2) | – |
| Ectopic pregnancies[∞] | – | 0.1(0–2) | – |
| Failed embryotransfers[∞] | – | 1.3(0–9) | – |

[#] Mean ± SD.
[∞] Mean (range).
[†] n(%).

## 4. Discussion

Our study has shown that LIF epithelial expression is significantly decreased in the endometrium of women with unexplained infertility, while comparable expression levels were observed between all other infertility sub-groups and the controls. In contrast, LIF-R expression was found significantly decreased in all sub-groups of infertile women. Furthermore, LIF-R stromal expression was significantly decreased in women with endometriosis.

Despite the fact that LIF expression has been examined in various studies, no definitive results have been observed on their expression patterns in various infertility sub-groups. There have been few studies comparing LIF expression between women with unexplained infertility and fertile controls. [10,16,17] Hambartsoumian et al. [16] observed impaired LIF epithelial expression by using ELISA, while Laird et al. [10] reported similar results by using real-time PCR. There has been no study that found comparable levels of LIF epithelial expression between fertile women and infertile women with unexplained infertility. However, our study reports on LIF expression levels by using IHC, which is a method that, despite its subjective character, reflects the actual protein expression levels in the endometrial tissue rather than mRNA levels. In summary, there are strong indications that LIF expression is decreased in the endometrial epithelial cells of women with unexplained infertility, implying an etiopathogenetic role for this cytokine in causing infertility, potentially through molecular impairment of the endometrium during the implantation window.

**Table 2**
LIF and LIF-R expression levels among various sub-groups of infertility.

| Parameters | Fertile (group 1) n = 20 | Tubal factor (group 2a) n = 15 | Poor ovarian reserve (group 2b) n = 20 | Endometriosis (group 2c) n = 10 | Unexplained infertility (group 2d)n = 15 |
|---|---|---|---|---|---|
| **LIF** | | | | | |
| *Epithelial cells* | | | | | |
| Positive nuclei percentage | 40.8 ± 6.8 | 29.3 ± 7.2 | 32.2 ± 7.7 | 31.6 ± 11.5 | 17.9 ± 3.5* |
| Intensity of staining | 2.4 ± 0.1 | 2.1 ± 0.2 | 1.9 ± 0.3 | 2.0 ± 0.2 | 1.8 ± 0.2* |
| h-score | 106.3 ± 19.6 | 69.0 ± 17.2 | 80.6 ± 21.4 | 79.6 ± 35.3 | 38.9 ± 9.9* |
| *Stromal cells* | | | | | |
| Positive nuclei percentage | 58.3 ± 5.2 | 62.7 ± 4.3 | 60.6 ± 4.7 | 64.0 ± 7.5 | 56.3 ± 4.0 |
| Intensity of staining | 2.4 ± 0.1 | 2.5 ± 0.1 | 2.4 ± 0.2 | 2.2 ± 0.2 | 2.4 ± 0.1 |
| h-score | 130.8 ± 17.0 | 153.3 ± 12.2 | 149.5 ± 17.2 | 149.0 ± 27.8 | 137.0 ± 13.6 |
| **LIF-R** | | | | | |
| *Epithelial cells* | | | | | |
| Positive nuclei percentage | 72.8 ± 4.2 | 60.3 ± 5.0* | 56.5 ± 6.2* | 40.0 ± 2.1* | 58.4 ± 5.8* |
| Intensity of staining | 2.6 ± 0.1 | 2.2 ± 0.2* | 2.1 ± 0.1* | 2.2 ± 0.1* | 2.3 ± 0.1* |
| h-score | 189.2 ± 14.4 | 136.8 ± 14.9* | 130.3 ± 9.1* | 91.0 ± 6.2* | 134.2 ± 14.3* |
| *Stromal cells* | | | | | |
| Positive nuclei percentage | 71.4 ± 3.0 | 75.7 ± 3.3 | 60.3 ± 4.2 | 59.0 ± 4.9* | 71.7 ± 3.0 |
| Intensity of staining | 2.6 ± 0.1 | 2.5 ± 0.1 | 2.3 ± 0.2 | 2.2 ± 0.1* | 2.6 ± 0.1 |
| h-score | 186.4 ± 9.9 | 188.5 ± 15.2 | 157.2 ± 15.1 | 137.5 ± 13.9* | 197.5 ± 11.0 |

* Significant difference between sub-group and control group of fertile women.

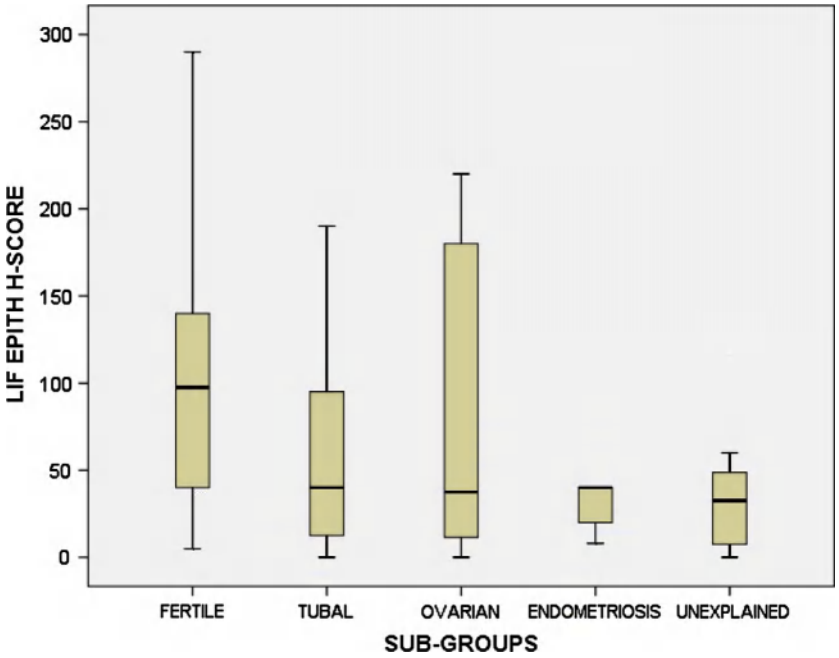


**Fig. 1.** Expression of LIF in epithelial cells of various sub-categories of infertile women.

A discrepancy is observed regarding LIF expression patterns in other infertility sub-groups. Dimitriadis et al. [9] have observed decreased expression levels in women with endometriosis, which was disputed by Mikolajczyk et al. [17]. To date, no study has examined LIF expression levels in women with poor ovarian reserve, while Aghajanova et al. have reported significantly impaired expression in women with tubal factor infertility [22]. Furthermore, there are several studies comparing a general infertile population with fertile controls that have reported controversial results concerning LIF expression levels in infertile women [23–26]. Potential explanations for this discrepancy may be the different methods used to compare expression levels within sub-groups. Thus, further research needs to be performed in order to assess the exact expression patterns of LIF in the various sub-categories of infertile women.

Whereas gp130 has been reported by many groups as showing impaired expression in infertile women, the LIF-Receptor alpha sub-unit has not received similar attention. As previously stated, the basic condition of LIF action is its connection with the LIF-Receptor as a primary step to create a high-affinity binding heterodimer. A search of the literature revealed few studies that directly compared LIF-R expression between fertile and infertile women during the implantation window. Thus, in a study performed on hamsters, Ding et al. underlined the significant role of LIF-R for uterine receptivity and implantation [13]. Further, Subramani et al. reported that LIF-R is dysregulated in women with dormant genital tuberculosis, potentially explaining the associated repeated implantation failure [27]. Linally, Moberg et al.

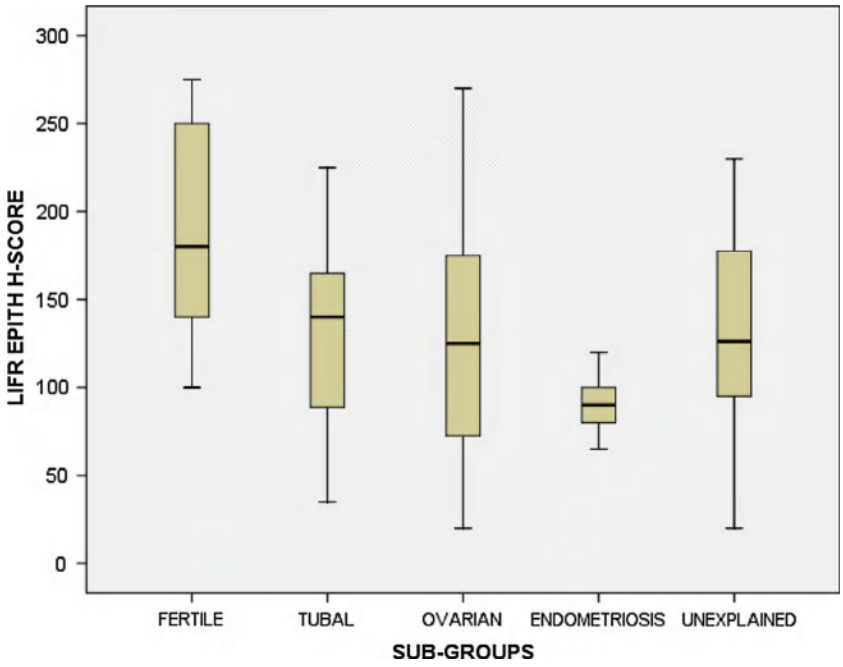


**Fig. 2.** Expression of LIF-R in epithelia cells of various sub-categories of infertile women.

reported that endometrial expression of the LIF-R was observed in women with endometriosis [28].

Our study is one of the few to report that LIF-R expression is significantly decreased in the epithelial endometrial cells of all infertile women. Moreover, the finding that LIF-R expression is significantly decreased in the cells of women with endometriosis should be further studied. In any case, as LIF-R levels are decreased in all sub-groups of infertile women, while LIF expression is only lower in women with unexplained infertility, it seems that the key to implantation may lie not in LIF expression but in the synchronized expression of adequate LIF-R.

The main clinical impact of our study may concern the management of cases with unexplained infertility. The exact etiopathogenetic drawback of these cases remains unclear. Impairment of certain molecules enrolled in implantation process may be a basic cause of fertility impairment in such cases. There could be hypothesized that disturbances in the molecular endometrial profiling result in a non-receptive endometrium. Detection of molecules presenting significant disturbances may permit the establishment of targeted therapies in an effort to restore their expression levels of before performing embryo transfer so that receptive endometrium is reassured. Such a prospective, however, necessitates much further research with large prospective studies including well-selected patients.

There are certain limitations in this study, the major one being the small sample size of its sub-groups. However, the majority of other published studies actually does not include much greater sample sizes given the nature of study that necessitated endometrial biopsy in a sensitive population of infertile women that are rather psychologically stressed and have already undergone multiple examinations. Besides, the overall sample size of infertile is 60 women, which is relatively one of the greater sample sizes included in relative studies. Furthermore, the current study is a prospective observational, but not matched for age between groups of fertile and infertile women. However, no significant correlation was detected between age and LIF, LIF-R expression in epithelial and stromal cells based on regression analysis, therefore no bias of age on final results may actually be justified. Besides, the majority of relative studies are not designed as matched for age because of profound difficulties in enrolling younger infertile women of all sub-groups of infertility. Furthermore, to the best of our knowledge, this is one of very few studies to report on LIF and LIF-R expression levels in all sub-categories of infertility, both in epithelial and stromal cells. Inclusion of an even larger number of patients and evaluation of LIF and LIF-R in the various infertility sub-groups will hopefully lead to safer conclusions regarding the potential etiopathogenetic role of these molecules in infertility.

In conclusion, our study indicated impaired LIF expression levels only in women with unexplained infertility, while LIF-R expression was

**Table 3**
Endometrial biopsy characteristics in various sub-fertility groups.

| Parameters | Fertile (group 1) n = 20 | Tubal factor (group 2a) n = 15 | Poor ovarian reserve (group 2b) n = 20 | Endometriosis (group 2c) n = 10 | Unexplained infertility (group 2d) n = 15 |
|---|---|---|---|---|---|
| Ovulation day[#] | 12.7 ± 1.4 | 14.0 ± 1.7* | 14.2 ± 1.9* | 13.8 ± 1.7* | 13.9 ± 1.7* |
| Menstrual day at biopsy[#] | 19.7 ± 1.8 | 21.3 ± 1.9* | 21.4 ± 2.0* | 21.0 ± 1.4* | 20.5 ± 2.8 |
| Ovulation-to-biopsy interval[#] | 7.1 ± 0.5 | 7.3 ± 0.5 | 7.1 ± 0.5 | 7.3 ± 0.5 | 7.1 ± 0.5 |
| Endometrial dating[#] | 17.6 ± 2.0 | 17.1 ± 2.4 | 18.1 ± 2.2 | 16.5 ± 2.1 | 17.4 ± 2.4 |
| Biopsy day-dating difference[∞] | −2.1 ± 2.0 | −4.1 ± 2.4* | −3.6 ± 2.3 | −5.7 ± 2.9* | −3.5 ± 4.3* |
| Out-of-phase tissues[†] | 4(20.0) | 10(66.7) * | 10(50.0) * | 6(60.0) * | 9(60.0) * |

[#] Mean ± SD.
[∞] Mean (range).
[†] n(%).
* Significant difference between sub-group and control group of fertile women.

impaired in all sub-groups of infertile women. Further, larger prospective studies are needed in order to assess the exact etiopathogenetic role of these cytokines in the molecular background of infertility.

## Conflict of interest

The authors report no conflict of interest.

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