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Removal of hydrosalpinges increases endometrial leukaemia inhibitory factor (LIF) expression at the time of the implantation window

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BACKGROUND: The presence of hydrosalpinges is associated with lower implantation and pregnancy rates in women undergoing IVF–embryo transfer, while salpingectomy improves these parameters. Although the mechanism by which hydrosalpinges affects fertility is not entirely understood, an adverse effect on endometrial receptivity has been postulated. In this study, we hypothesized that the adverse effects of hydrosalpinges on fertility may be in part mediated by inappropriate endometrial expression of leukaemia inhibitory factor (LIF), a cytokine implicated in implantation. METHODS: In order to test our hypothesis, we prospectively examined the expression of LIF during the window of implantation in the endometrium of infertile women ($n = 10$) with hydrosalpinges prior to and following salpingectomy and of fertile controls ($n = 10$) by Western blotting and immunohistochemistry. RESULTS: LIF expression was significantly lower in infertile women with hydrosalpinges compared with fertile controls ($P < 0.05$). Salpingectomy resulted in an increase in LIF expression in eight out of 10 women with hydrosalpinges. LIF levels were increased by 231 ± 49% (mean ± SEM) following salpingectomy. Immunohistochemical analysis confirmed the Western blot findings. The increased LIF immunoreactivity was predominantly localized to luminal and glandular epithelial cells. CONCLUSIONS: Our findings suggest that observed benefit from salpingectomy in infertile women with hydrosalpinges may be in part mediated by the up-regulation of endometrial LIF expression.

Key words: endometrial receptivity/hydrosalpinx/implantation/leukaemia inhibitory factor/salpingectomy

Introduction

Implantation is a complex process by which the embryo attaches to the endometrium, first penetrating the endometrial epithelium and then invading the maternal circulatory system to form the placenta (Giudice, 1999). Timely modifications in the endometrium to become receptive to the developing embryo are crucial for successful implantation (Navot *et al.*, 1991). The human endometrium becomes receptive to the embryo only for a limited period during the luteal phase of the menstrual cycle, under the influence of steroid hormones and paracrine factors originating from endometrial cells and the embryo (Cavagna and Mantese, 2003). The period during which endometrium is receptive to implantation, termed the implantation window, begins approximately 6 days after ovulation and is believed to encompass cycle days 20–24 (Lessey, 2000).

Impaired endometrial receptivity is considered to be a major limiting factor for the establishment of pregnancy (Edwards, 1995). In an attempt to develop a clinically relevant and reproducible evaluation of endometrial function, a number of molecular and morphological markers specific to the implantation window have been identified. These include pinopodes, integrins, leukaemia inhibitory factor (LIF), the interleukin-1 system, glycodelin, colony stimulating factor-1, heparin-binding epidermal growth factor, and the HOX genes (Cavagna and Mantese, 2003; Giudice, 1999; Taylor *et al*., 1997, 1998). Although these markers have been shown to be essential for implantation in animal models, further studies are needed to reveal their roles in human implantation (Cavagna and Mantese, 2003; Lessey, 2000).

LIF, a member of the interleukin (IL)-6-type cytokine family, is one of the potential markers of endometrial receptivity (Senturk and Arici, 1998). LIF, initially identified by its ability to induce the differentiation of the myeloid leukaemia cell line M1, has multiple biological activities in many different cell types, including proliferation, differentiation and cell survival (Gearing *et al.*, 1987; Senturk and Arici, 1998). A role for LIF

in implantation was first demonstrated by studies in which transgenic mice homozygous-deficient for LIF could produce normal embryos, but implantation failed to occur (Stewart *et al.*, 1992). In human endometrium, LIF is expressed only at very low levels during the proliferative phase, while both LIF protein and mRNA are expressed abundantly in the luminal and glandular epithelium during the middle and late secretory phases (Arici *et al.*, 1995; Vogiagis *et al.*, 1996). LIF levels in the uterine flushing fluid are significantly higher in fertile women compared to women with unexplained infertility (Laird *et al.*, 1997). Moreover, LIF secretion in endometrial explant cultures obtained from fertile women on days 18–21 of the cycle is greater than in cultures from women with unexplained infertility or with multiple failures of implantation (Hambartsoumian, 1998). These studies are supported by findings of mutations in the coding region of the LIF gene in some infertile women (Steck *et al.*, 2004) and argue that the timely increase in endometrial LIF expression during the implantation window plays an important role in implantation.

Hydrosalpinx is described as a distally blocked, dilated, fluid-filled fallopian tube with a heterogeneous spectrum of pathology. Two meta-analyses have shown that women with hydrosalpinx have lower implantation, pregnancy and delivery rates, and a higher incidence of spontaneous abortion after IVF–embryo transfer compared with women with tubal infertility of other causes (Zeyneloglu *et al.*, 1998; Camus *et al.*, 1999). Furthermore, a prospective randomized clinical trial and a Cochrane review have demonstrated improved pregnancy and delivery rates with laparoscopic salpingectomy for hydrosalpinges prior to IVF (Strandell *et al.*, 2001; Johnson *et al.*, 2002). These findings suggest that, besides occluding the fallopian tubes, hydrosalpinx may also affect infertility through other mechanisms. One theory to explain the deleterious effect of a hydrosalpinx on the outcome of IVF is the intermittent bathing of the intrauterine environment with toxic fluid within the hydrosalpinx. The hydrosalpinx fluid may mechanically interfere with the apposition of the implanting embryo (Mansour *et al.*, 1991) or may impede embryo development due to its deficiencies in essential factors (Strandell *et al.*, 1998). The presence of hydrosalpinx may also reduce the receptivity of the endometrium by decreasing the expression of specific factors. One such factor is $\alpha_v\beta_3$ integrin, the expression of which has been shown to be decreased in the endometrium of women with hydrosalpinx and to be increased following salpingectomy during the window of implantation (Meyer *et al.*, 1997; Bildirici *et al.*, 2001).

In this prospective study, we hypothesized that the adverse effects of hydrosalpinges on fertility may be in part mediated by inappropriate expression of another endometrial receptivity marker, LIF. In order to test our hypothesis, we examined the expression of LIF in the endometrium of infertile women with hydrosalpinges at the time of the implantation window prior to and following salpingectomy.

Materials and methods

Tissue collection

Infertile women with unilateral or bilateral communicating hydrosalpinges detected by hysterosalpingography were evaluated for possible inclusion in the study during a 2-year period. Among these subjects, women younger than 40 years and those having regular menstrual cycles with no ovulatory dysfunction (mid-luteal progesterone >10 ng/ml) were included. Male factor infertility was excluded by the presence of normal semen parameters according to the World Health Organization definition (sperm count $>20 \times 10^6$/ml, progressive motility >40%, normal sperm morphology >40%) (World Health Organization, 1992). This study was approved by the Institutional Review Board of Macettepe University School of Medicine.

After all the patients had been informed and written consent obtained, they were assigned to laparoscopy scheduled during the putative window of implantation (cycle days 19–21). The day of the menstrual cycle was established from the patient's menstrual history. Following laparoscopic confirmation of hydrosalpinges and associated severe tubal disease, salpingectomy and endometrial sampling were performed in 10 patients. These women were subjected to endometrial sampling on the corresponding menstrual cycle days of the fourth post-treatment cycle. The Pipelle device (Laboratoire CCD, Paris, France) was used for all biopsies. Endometrial samples obtained during cycle days 19–21 from 10 age-matched women with proven fertility undergoing non-fertility-related surgery were used as controls in the study. These women had regular menses and had no known medical problems. They all had at least one successful pregnancy in the past.

Western blotting

Total protein from the endometrial tissues were extracted using T-PER tissue protein extraction reagent (Pierce, Rockford, IL, USA), supplemented with protease inhibitor cocktail (1 mM Na_3VO_4, 10 μg/ml leupeptin, 10 μg/ml aprotinin and 1 mM phenylmethylsulphonylfluoride; Calbiochem, San Diego, CA, USA). The protein concentration was determined by detergent-compatible Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Western blot analysis was performed as described previously (Guzeloglu-Kayisli *et al.*, 2004). Briefly, 20 μg of protein was loaded into each lane, separated electrophoretically by SDS–PAGE using 10% Tris–HCl Ready Gels (Bio-Rad Laboratories), and electroblotted onto nitrocellulose membrane (Bio-Rad Laboratories). The membrane was blocked with 5% non-fat dry milk in TBS-T buffer (0.1% Tween-20 in Tris-buffered saline) for 1 h to reduce the non-specific binding. The membrane was then incubated with goat polyclonal anti-human LIF antibody (1:400 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C, and washed three times with TBS-T for 20 min. Then, the membrane was incubated for 1 h with peroxidase-labelled anti-goat IgG (Vector Laboratories, Burlingame, CA, USA) and subsequently washed with TBS-T three times for 20 min. LIF immunoreactivity was detected using chemiluminescent detecting reagents (Perkin Elmer Life Sciences, Boston, MA, USA) and exposure of the membrane to BioMax film (Kodak, Rochester, NY, USA).

After the membrane had been stripped with stripping solution (Pierce), the same membrane was reprobed with mouse monoclonal antihuman glyceraldehyde-3-phosphate dehydrogenase antibody (GAPDH; Santa Cruz Biotechnology). Immunoblot bands for LIF and GAPDH were quantified using a laser densitometer. Each LIF band was normalized to the value obtained from the corresponding GAPDM band.

Immunohistochemistry

The endometrial samples were transported on ice, embedded in OCT (Tissue Tek, Torrance, CA, USA), snap-frozen in liquid nitrogen and kept at –80°C until use. Serial cryosections (thickness 5 μm) were placed on poly-L-lysine-coated microscope slides and fixed at +4°C in acetone for 10 min. Sections were rinsed twice in phosphate-buffered

saline (PBS; pH 7.4) for 5 min each and in PBS with bovine serum albumin (PBS-BSA; 0.1% wt/vol) for 10 min. Endogenous peroxidase activity was quenched with 3% H_2O_2 in PBS for 15 min. Slides were then incubated with 5% blocking horse serum (Vector Laboratories) for 1 h at room temperature in a humidified chamber. Excess serum was drained, and primary antibody (goat polyclonal anti-human LIF antibody; Santa Cruz Biotechnology; 1:40 dilution in PBS-BSA) was added to the sections. For the negative control, normal goat IgG was used at the same concentration. Sections were incubated overnight at 4°C in a humidified chamber, rinsed, and then treated with biotinylated horse anti-goat antibody (Vector Laboratories) at 1:250 dilution for 30 min at room temperature. The antigen–antibody complex was detected by using an avidin–biotin–peroxidase kit (ABC; Vector Laboratories). Subsequently, the chromogenic reaction was carried out with 3-amino 9-ethyl carbazole (Vector Laboratories) and the reaction was terminated with tap water. Slides were counterstained with haematoxylin prior to permanent mounting and then evaluated under a light microscope. One slide for each case was also stained with haematoxylin and eosin for endometrial histological dating, according to the criteria of Noyes and colleagues (Noyes *et al.*, 1975).

The intensity of LIF immunoreactivity in endometrial tissues was evaluated semiquantitatively using the following intensity categories: 0, no staining; 1+, weak but detectable staining; 2+, moderate or distinct staining; and 3+, intense staining. For each tissue, a HSCORE value was derived by summing the percentages of cells that stained at each intensity category and multiplying that value by the weighted intensity of the staining, using the formula $\text{HSCORE} = \Sigma P_i(i + 1)$, where i represents the intensity scores and P_i is the corresponding percentage of the cells. In each slide, five different areas and 100 cells in each area were evaluated under a microscope with a ×40 objective, the percentage of cells for each intensity within these areas was determined at different times by two investigators blinded to the source of the samples, and the average score was then used.

Statistical analysis

Since the data from immunohistochemistry and Western blot analysis were normally distributed (as determined with the Kolmogorov–Smirnov test), comparisons of samples were analysed with Student's *t*-test or paired *t*-test when appropriate. Statistical calculations were performed using SigmaStat for Windows, version 3.0 (Jandel Scientific, San Rafael, CA, USA). Statistical significance was defined as $P < 0.05$.

Results

The clinical characteristics of infertile patients (n = 10) with hydrosalpinges diagnosed by hysterosalpingography are summarized in Table I. The mean age of the patients was 30.1 ± 4.0 (mean ± SD) years. These patients had been trying to conceive for an average of 6.7 ± 3.6 years. Their mean basal FSH level was 5.4 ± 2.1 mIU/ml. Six of these 10 patients had bilateral hydrosalpinges and the rest had unilateral hydrosalpinx. Transvaginal ultrasound performed prior to surgery revealed visible hydrosalpinges in five out of 10 women. Histopathological evaluation of all salpingectomy specimens confirmed chronic salpingitis with distal tubal occlusion and hydrosalpinx, irrespective of ultrasound visibility. The mean age of the fertile (control) patients was 31.4 ± 4.2 (mean ± SD) years. The conventional histological dating of endometrial biopsies was in concordance with the chronological dating in all cases. Thus, no out-of-phase endometrium in either controls or pre- or post-salpingectomy samples was observed.

Table I. Demographic characteristics of infertile patients with hydrosalpinges

Patient number	Age (years)	Duration of infertility (years)	Visibility by ultrasonography	Involvement
1	34	14	+	Bilateral
2	29	8	–	Unilateral
3	35	3	+	Unilateral
4	24	4	–	Bilateral
5	33	5	–	Unilateral
6	29	4	+	Bilateral
7	33	10	–	Bilateral
8	32	5	–	Bilateral
9	24	4	+	Unilateral
10	28	10	+	Bilateral

In order to evaluate the effect of hydrosalpinx on endometrial LIF expression during the implantation window, LIF levels in endometrial samples from infertile patients with hydrosalpinges (n = 10) were first compared with their age-matched fertile controls (n = 10) by Western blot analysis. Endometrial LIF expression during the window of implantation was significantly lower in women with hydrosalpinges compared to fertile controls (P = 0.007; Figure 1A).

Next, we assessed the influence of salpingectomy on LIF expression by comparing pre- and post-operative endometrial samples of the same patient by Western blot analysis (Figure 1B). We observed an increase in LIF expression in eight of the 10 post-salpingectomy endometrial samples (Figure 1C). LIF levels were increased by 231 ± 49% (mean ± SEM) following salpingectomy after normalization with GAPDH (P = 0.011; Figure 1A). When we compared the endometrial LIF levels of the post-salpingectomy samples with their age-matched fertile controls, we did not observe any significant difference (Figure 1A).

In order to determine the localization of LIF expression, endometrial tissues sampled before and after the salpingectomy were also evaluated by immunohistochemistry. Immunohistochemical results revealed that both luminal and glandular epithelial cells abundantly expressed LIF in all samples (Figure 2A–D). Diffuse cytoplasmic and membranous staining patterns were noticed. The stromal component of the endometrium showed weaker staining compared with the epithelial cells in both pre- and post-salpingectomy samples. Eight out of 10 cases showed an increase in total HSCORE after salpingectomy (Figure 2E). The HSCORE value of the LIF staining was significantly increased by 216 ± 35% (mean ± SEM) in post-salpingectomy endometrial samples compared with pre-salpingectomy endometrial samples of the same patient (P = 0.004).

Discussion

In this study, we have demonstrated that the expression of LIF in the endometrium during the implantation window is significantly lower in infertile women with hydrosalpinges compared to fertile controls, and that LIF expression is increased at the time of the implantation window after salpingectomy, suggesting an improvement in endometrial receptivity. Our findings support previous reports about the detrimental effects of hydrosalpinx

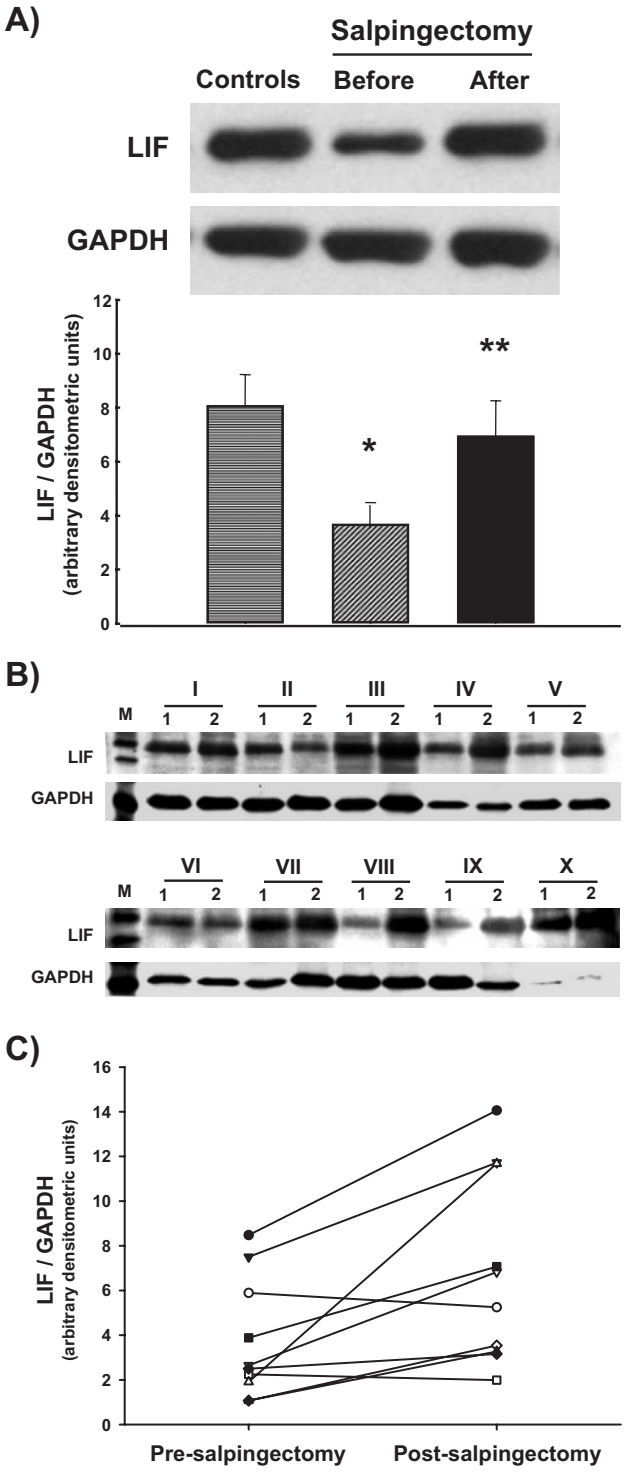

Figure 1. (A) Western blot analysis of LIF protein in the endometrium of infertile women (n = 10) with hydrosalpinges before and after salpingectomy, and of age-matched fertile controls (n = 10). LIF levels were normalized to GAPDH protein level. Representative blot is presented. Graph represents mean ± SEM. *P = 0.007 versus control; **P = 0.011 versus before salpingectomy. (B) Western blot analysis of LIF protein in the endometrium of infertile women (n = 10) with hydrosalpinges before (1) and after (2) salpingectomy. M = molecular weight marker. Roman numbers represent patient numbers. (C) Scattergram of Western blot analysis of LIF protein in the endometrium of infertile women (n = 10) with hydrosalpinges before and after salpingectomy. LIF levels were normalized to GAPDH protein level. LIF protein expression increased in eight of the 10 post-salpingectomy endometrial samples.

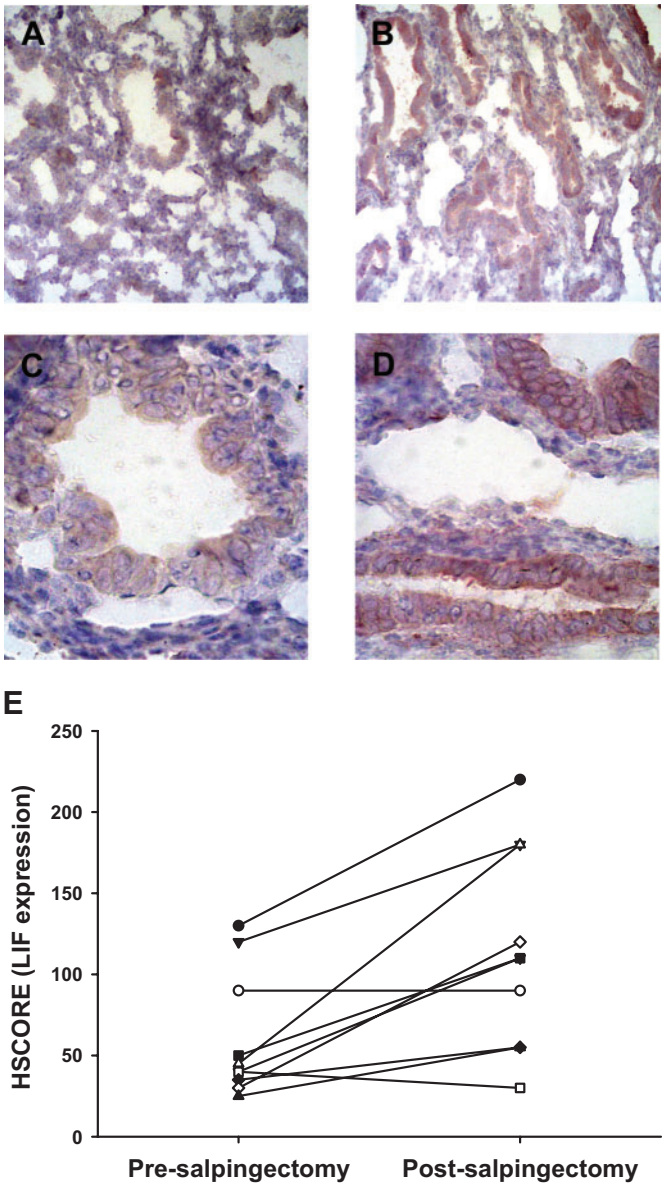

Figure 2. Immunolocalization of LIF in the endometrium from infertile women with hydrosalpinges before (A, C) and after (B, D) salpingectomy. LIF immunoreactivity improved after salpingectomy in infertile women with hydrosalpinges and was mostly localized in the glands. Original magnification: A, B ×25; C, D ×100. (E) Scattergram of LIF immunostaining intensity (HSCORE) in the endometrium of infertile women (n = 10) with hydrosalpinges before and after salpingectomy. Eight out of 10 cases showed an increase in the total HSCORE after salpingectomy.

on implantation and the benefit of its surgical removal on endometrial receptivity.

Meta-analyses have shown that women with hydrosalpinges have about half the implantation and pregnancy rates in IVF–embryo transfer compared with women with tubal infertility of other causes (Zeyneloglu *et al.*, 1998; Camus *et al.*, 1999). One of the explanations for the hydrosalpinx-related decrease in pregnancy rates suggests that leakage of hydrosalpinx fluid into the uterine cavity creates an unfavourable endometrial environment for implantation (Kodaman *et al.*, 2004). According to this theory, prevention of this leakage should improve the

implantation and pregnancy rates. In this study, laparoscopic salpingectomy was performed in infertile patients with hydrosalpinges. While other techniques, including proximal tubal ligation, neosalpingostomy and ultrasound-guided transvaginal aspiration of vaginal fluid, are also reported to prevent the hazardous effects of hydrosalpinx fluid, the data demonstrating their effectiveness on implantation and pregnancy rates are limited and controversial (Hammadieh *et al.*, 2004).

The post-salpingectomy endometrial biopsies were performed arbitrarily in the fourth post-salpingectomy menstrual cycle. This strategy was employed as the normalization of endometrium has been accepted as happening after three cycles of continued treatment in certain disorders, such as dysfunctional uterine bleeding (Speroff and Fritz, 2005). Performing sequential monthly biopsies to determine the progressive change would have been ideal, but this was not possible due to ethical concerns. Therefore, the timing of optimal improvement in endometrial receptivity markers following salpingectomy remains undetermined.

Endometrial biopsy specimens contain several cell populations, including epithelial, stromal and endothelial cells. The established methods of endometrial biopsy used in this study are performed without direct visualization, and sample-to-sample variation in the epithelial/stromal cell ratio would be anticipated. Since LIF protein is expressed predominantly in endometrial epithelial cells, variations in epithelial/stromal cell ratio could affect the detected LIF expression using Western blot analysis. Although determination of LIF protein expression could be attempted after laser capture microdissection of endometrial epithelial cells, obtaining insufficient protein for Western analysis precludes the use of this technique. Therefore, we performed a second technique, immunohistochemistry, to confirm results of Western analysis and to determine the localization of increased LIF expression in the endometrium.

Endometrial receptivity is the temporally and spatially regulated set of circumstances within the endometrium that facilitates successful embryonic implantation (Giudice, 1999). Although the endometrial stroma may also play a role, endometrial receptivity is mostly attributed to the endometrial epithelium (Giudice, 1999). In this study, we observed higher LIF protein expression in endometrial epithelial cells compared with the stroma. Our findings are consistent with those of previous studies (Cullinan *et al.*, 1996; Vogiagis *et al.*, 1996). Furthermore, following salpingectomy, an increase in LIF expression was observed predominantly in the luminal and glandular epithelium. Although the expression pattern of LIF in the endometrium is well established, it is not clear how LIF specifically functions in implantation. Besides the constitutive expression of LIF receptor (LIFR) in the endometrial luminal epithelium (Cullinan *et al.*, 1996), LIFR transcripts were also detected in human pre-implanting embryos, suggesting the embryo as a possible target (Cullinan *et al.*, 1996). LIF may also regulate embryonic implantation by direct modulation of trophoblast differentiation from the cytotrophoblast towards an anchoring extravillous phenotype (Nachtigall *et al.*, 1996). Previously, using the same specimens, Bildirici and colleagues (2001) have reported an improvement in the expression of endometrial $\alpha_v\beta_3$ integrin following salpingectomy in women with hydrosalpinges. We would speculate that an upstream factor such as HOXA10 or HOXA11 may be regulating both $\alpha_v\beta_3$ integrin and LIF expression in the endometrium.

A role for LIF has also been implicated in the outcome of assisted reproductive techniques (ART). Low endometrial concentrations of LIF protein during the window of implantation are associated with unexplained infertility and a high risk of implantation failure after embryo transfer (Laird *et al.*, 1997; Hambartsoumian, 1998). Recently, treatment with recombinant human LIF prior to embryo transfer has been shown to improve pregnancy rates in women with a history of recurrent implantation failure (Brinsden *et al.*, 2003). We speculate that a decrease in LIF expression may be a mediator of the adverse effects of hydrosalpinges on fertility, and that improvement in IVF outcome following salpingectomy in women with hydrosalpinges may be due in part to an increase in endometrial LIF expression. Further studies will be needed to clarify the relative importance of endometrial LIF expression in ART outcome, and to determine whether LIF may be effectively used in order to improve pregnancy rates in women undergoing IVF–embryo transfer. Moreover, if LIF is to be used therapeutically, it will be necessary to determine the appropriate patient population, and to develop laboratory techniques to identify them.

References

Arici A, Engin O, Attar E and Olive DL (1995) Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in human endometrium. J Clin Endocrinol Metab 80,1908–1915.

Bildirici I, Bukulmez O, Ensari A, Yarali H and Gurgan T (2001) A prospective evaluation of the effect of salpingectomy on endometrial receptivity in cases of women with communicating hydrosalpinges. Hum Reprod 16,2422–2426.

Brinsden PR, Ndukwe G, Engrand P, Pinkstone S, Lancaster S, Macnamee MC and De Moustier B (2003) Does recombinant human leukemia inhibitory factor improve implantation in women with recurrent failure of assisted reproduction treatment? [Abstract]. In: 19th Annual Meeting of the European Society for Human Reproduction and Embryology, Madrid, Spain, p. 18.

Camus E, Poncelet C, Goffinet F, Wainer B, Merlet F, Nisand I and Philippe HJ (1999) Pregnancy rates after in-vitro fertilization in cases of tubal infertility with and without hydrosalpinx: a meta-analysis of published comparative studies. Hum Reprod 14,1243–1249.

Cavagna M and Mantese JC (2003) Biomarkers of endometrial receptivity—a review. Placenta 24 Suppl B,S39–S47.

Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA and Stewart CL (1996) Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. Proc Natl Acad Sci USA 93,3115–3120.

Edwards RG (1995) Clinical approaches to increasing uterine receptivity during human implantation. Hum Reprod 10 Suppl 2,60–66.

Gearing DP, Gough NM, King JA, Hilton DJ, Nicola NA, Simpson RJ, Nice EC, Kelso A and Metcalf D (1987) Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). EMBO J 6,3995–4002.

Giudice LC (1999) Potential biochemical markers of uterine receptivity. Hum Reprod 14 Suppl 2,3–16.

Guzeloglu-Kayisli O, Kayisli UA, Luleci G and Arici A (2004) *In vivo* and *in vitro* regulation of Akt activation in human endometrial cells is estrogen dependent. Biol Reprod 71,714–721.

Hambartsoumian E (1998) Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. Am J Reprod Immunol 39,137–143.

Hammadieh N, Afnan M, Evans J, Sharif K, Amso N and Olufowobi O (2004) A postal survey of hydrosalpinx management prior to IVF in the United Kingdom. Hum Reprod 19,1009–1012.

Johnson NP, Mak W and Sowter MC (2002) Laparoscopic salpingectomy for women with hydrosalpinges enhances the success of IVF: a Cochrane review. Hum Reprod 17,543–548.

Kodaman PH, Arici A and Seli E (2004) Evidence-based diagnosis and management of tubal factor infertility. Curr Opin Obstet Gynecol 16,221–229.

Laird SM, Tuckerman EM, Dalton CF, Dunphy BC, Li TC and Zhang X (1997) The production of leukaemia inhibitory factor by human endometrium: presence in uterine flushings and production by cells in culture. Hum Reprod 12,569–574.

Lessey BA (2000) The role of the endometrium during embryo implantation. Hum Reprod 15 Suppl 6,39–50.

Mansour RT, Aboulghar MA, Serour GI and Riad R (1991) Fluid accumulation of the uterine cavity before embryo transfer: a possible hindrance for implantation. J In Vitro Fert Embryo Transf 8,157–159.

Meyer WR, Castelbaum AJ, Somkuti S, Sagoskin AW, Doyle M, Harris JE and Lessey BA (1997) Hydrosalpinges adversely affect markers of endometrial receptivity. Hum Reprod 12,1393–1398.

Nachtigall MJ, Kliman HJ, Feinberg RF, Olive DL, Engin O and Arici A (1996) The effect of leukemia inhibitory factor (LIF) on trophoblast differentiation: a potential role in human implantation. J Clin Endocrinol Metab 81,801–806.

Navot D, Scott RT, Droesch K, Veeck LL, Liu HC and Rosenwaks Z (1991) The window of embryo transfer and the efficiency of human conception *in vitro*. Fertil Steril 55,114–118.

Noyes RW, Hertig AT and Rock J (1975) Dating the endometrial biopsy. Am J Obstet Gynecol 122,262–263.

Senturk LM and Arici A (1998) Leukemia inhibitory factor in human reproduction. Am J Reprod Immunol 39,144–151.

Speroff L and Fritz MA (2005) Clinical gynecologic endocrinology and infertility, 7th edn. Philadelphia: Lippincott Williams & Wilkins.

Steck T, Giess R, Suetterlin MW, Bolland M, Wiest S, Poehls UG and Dietl J (2004) Leukaemia inhibitory factor (LIF) gene mutations in women with unexplained infertility and recurrent failure of implantation after IVF and embryo transfer. Eur J Obstet Gynecol Reprod Biol 112,69–73.

Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F and Abbondanzo SJ (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 359,76–79.

Strandell A, Sjogren A, Bentin-Ley U, Thorburn J, Hamberger L and Brannstrom M (1998) Hydrosalpinx fluid does not adversely affect the normal development of human embryos and implantation *in vitro*. Hum Reprod 13,2921–2925.

Strandell A, Lindhard A, Waldenstrom U and Thorburn J (2001) Hydrosalpinx and IVF outcome: cumulative results after salpingectomy in a randomized controlled trial. Hum Reprod 16,2403–2410.

Taylor HS, Vanden Heuvel GB and Igarashi P (1997) A conserved Hox axis in the mouse and human female reproductive system: late establishment and persistent adult expression of the Hoxa cluster genes. Biol Reprod 57,1338–1345.

Taylor HS, Arici A, Olive D and Igarashi P (1998) HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. J Clin Invest 101,1379–1384.

Vogiagis D, Marsh MM, Fry RC and Salamonsen LA (1996) Leukaemia inhibitory factor in human endometrium throughout the menstrual cycle. J Endocrinol 148,95–102.

World Health Organization (1992) WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 3rd edn. Cambridge, UK: Cambridge University Press.

Zeyneloglu HB, Arici A and Olive DL (1998) Adverse effects of hydrosalpinx on pregnancy rates after *in vitro* fertilization-embryo transfer. Fertil Steril 70,492–499.

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