Synthesis and regulation of leukaemia inhibitory factor in cultured bovine oviduct cells by hormones

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Leukaemia inhibitory factor (LIF) is an essential factor for embryo implantation. Factors generated by the oviduct cells (epithelial cells and fibroblasts) create the microenvironment for fertilization and first embryo stage development. Hence, it is feasible that the oviduct cells also synthesize LIF to promote and condition the embryo for implantation in the uterus. In the present study, we investigated whether cultured bovine oviduct epithelial cells and fibroblasts synthesize LIF. LIF production was measured in the conditioned medium of oviduct epithelial cells and fibroblasts, using LIF enzyme-linked immunosorbent assay. Moreover, expression of LIF mRNA was confirmed by LIF reverse transcriptase–polymerase chain reaction in extracts of RNA from oviduct epithelial/fibroblast cells. Quantitatively similar amounts of LIF were detected in the culture medium of epithelial cells and fibroblasts. In cells cultured for 1–7 days, the levels of LIF in the medium increased in a time-dependent manner. As compared to untreated cells, treatment of cells with 17β-oestradiol (1–100 ng/ml), but not progesterone (1–100 ng/ml) and insulin (20 ng/ml), increased the levels of LIF in a concentration-dependent manner (P < 0.05). Similarly, tumour necrosis factor-α (100 ng/ml) significantly induced the levels of LIF. The effects of 17β-oestradiol (50 ng/ml) on LIF synthesis were enhanced and not blocked in the presence of tamoxifen (1 µg/ml), an oestrogen receptor antagonist, suggesting that the stimulatory effects of 17β-oestradiol on LIF synthesis are not receptor-mediated. In conclusion 17β-oestradiol, but not progesterone, induces LIF synthesis by bovine oviduct epithelial cells and fibroblasts and this may play an important role in the biology of early embryo development. However, the exact pathophysiological role of LIF within the oviduct needs to be further investigated.

Key words: embryo/leukaemia inhibitory factor/17β-oestradiol/oviduct/tamoxifen

Introduction

Leukaemia inhibitory factor (LIF) is a 45–56 kDa glycoprotein (gp), first recognized as a haematopoietic regulator due to its ability to induce the differentiation of a murine myeloid leukaemic cell line M1 to macrophages (Tomida et al., 1984). Subsequently, LIF has been shown to be a pleiotropic molecule involved in different activities in various tissues and cell types (Hilton, 1992). LIF shares close homology with other cytokines, including interleukin 6 (IL-6), oncostatin M and granulocyte-colony stimulating factor. The biological effects of LIF are mediated via a receptor that consists of a low affinity (kD 1.0 nM) LIF binding subunit and a gp130 subunit. Moreover, oncostatin mediates its effects by binding to the LIF receptor, whereas gp130 is involved in the signalling pathway for IL-6 and IL-1. Together, these findings suggest that LIF shares a common structurally related signal transduction pathway with other cytokines and has overlapping biological activities (Gearing et al., 1991; Thoma et al., 1994).

In the reproductive system LIF is known to be an essential factor for the embryo implantation process. LIF-deficient male mice are fertile and LIF-deficient female mice undergo ovulation. Embryos in these mice develop to blastocysts but later fail to implant. Additionally, transplantation of blastocysts from LIF-deficient mice to a wild type results in term pregnancy (Stewart et al., 1992). Additionally, in the murine uterus, LIF is maximally expressed 4 days after fertilization, i.e. the day of implantation (Bhatt et al., 1991). Taken together these findings provide strong evidence for an essential role of LIF in the implantation process (Stewart et al., 1994).

LIF inhibits the differentiation of cytotrophoblastic cells towards an invasive phenotype by inhibiting the secretion of metalloproteinase (Bischof et al., 1995). Moreover, it markedly decreases the production of human chorionic gonadotrophin (hCG) by the trophoblast and increases oncofetal fibronectin mRNA expression and protein secretion (Nachtigall et al., 1996), biochemical changes that are characteristic of cytotrophoblast differentiation. Since LIF has also been detected in follicular fluid and its concentration is positively correlated with that of 17β-oestradiol (Arici et al., 1997) it could be that LIF is not only involved in the differentiation processes/ pathways occurring during the early stages of pregnancy but also in the oocyte maturation stage.

Expression of receptor transcript for LIF has been identified
in human oocytes and human embryos. However, no mRNA encoding LIF has been observed in preimplantation embryonic stages (Sharkey et al., 1995; Van Eijk et al., 1996). This finding supports the hypothesis that the effects of LIF have to be of maternal origin. Indeed, co-culture with cells that express LIF has been shown to enhance mouse blastocyst formation and development in vitro (Kauma and Matt, 1995). Moreover, LIF improves the viability of cultured bovine embryos (Fry et al., 1992).

LIF therefore plays an essential role in the implantation process and has been shown to have an effect in vitro on the developing embryo. The first stage of embryo development (prior to its transport to the uterus) occurs within the oviduct. We therefore postulate that LIF is synthesized within the oviduct and may play a key role in the physiological and pathological processes associated with implantation, the latter including ectopic pregnancy or failed implantation in the uterus. Moreover, we hypothesize that the synthesis of LIF is regulated by ovarian hormones and cytokines, such as tumour necrosis factor-α (TNF-α), factors that are actively involved in the implantation process.

Accordingly the aims of the present study were to determine: (i) whether bovine oviduct epithelial cells and fibroblasts synthesize LIF; (ii) whether synthesis of LIF by these two cell types is differential; (iii) whether ovarian hormones including 17β-oestradiol and progesterone regulate the synthesis of LIF and factors such as TNFα and insulin increase its synthesis by epithelial cells and fibroblasts; and (iv) whether the effects of 17β-oestradiol on LIF synthesis are receptor-operated.

Materials and methods

Isolation and culture of bovine oviduct cells

Mixed culture, epithelial cells and fibroblasts 1:1

Oviducts obtained from young cyclic non-pregnant cows were placed and the cell pellet washed twice with HBSS. The final pellet was collected in a sterile tube. The cell suspension obtained was centrifuged at room temperature. Following incubation, the oviducts were perfused with 0.5 mM EDTA, 5 mM HEPES; pH 7.4) and incubated for 30 min at 30°C in a water bath. The oviducts were then cut longitudinally and placed in a Petri dish and the luminal surface was scraped with a cell scraper. The cell suspension obtained in the Petri dish after the scraping was washed twice by centrifuging cells suspended in HBSS. The final cell pellet was suspended in complete culture medium and plated. After 24 h of incubation under standard tissue culture conditions, the supernatant was aspirated and the cells provided with fresh complete culture medium and allowed to grow to confluence. Cultures in the second passage were characterized to assess the purity of the culture and used for the study.

Cell characterization

Purity of both cultures, primary as well as first passage, was analysed by morphological as well as immunochemical assays as previously described (Rosselli et al., 1994). Monoclonal antibodies to epithelial cell cytotkeratin (anti-cytokeratin AE1/AE3, Dako Diagnostics AG, Zug, Switzerland) were used to the proportion of epithelial cells in all cultures, whereas an antibody against fibroblast vimentin (anti-vimentin VIM 3B4, Dako) was used to identify the proportion of fibroblasts in all cultures. Peroxidase–antiperoxidase staining (Dako) was used to visualize the immunoreactivity. Cell nuclei were counterstained with Harris haematoxylin (Merck, Darmstadt, Germany).

LIF synthesis

Subconfluent monolayers of mixed cultures from the bovine oviduct were washed with HBSS and fed Dulbecco's modified Eagle's medium (DMEM/Ham’s F12 (Gibco) supplemented with 1% charcoal-stripped FCS (Sigma, Buchs, Switzerland) for 4 days. The culture medium collected after 4 days was used to assay the presence of immunoreactive LIF. Moreover, to investigate whether the synthesis of LIF was time-dependent, the conditioned medium from a mixed culture was collected after 1, 3, 5 and 7 days of culture and the levels of LIF assayed.

In order to evaluate the regulation of the LIF synthesis, mixed cultures were treated 4 days with DMEM/Ham’s F12 (Sigma) supplemented with 1% charcoal-stripped FCS and containing or lacking: (i) 17β-oestradiol (1–100 ng/ml; Sigma); (ii) progesterone (1–100 ng/ml; Sigma); (iii) TNFα (100 ng/ml; Pepro Tech, Inc. Rocky Hill, NJ, USA); (iv) insulin (20 ng/ml; Sigma); (v) 17β-oestradiol (50 ng/ml) plus cycloheximide (1 μM), a protein synthesis inhibitor; (vi) 17β-oestradiol (50 ng/ml) plus EDTA (0.1 mM; Sigma), a calcium chelator; (vii) tamoxifen (10 ng/ml, 1 μg/ml; Sigma), a non-steroidal oestrogen receptor antagonist; and (viii) 17β-oestradiol (50 ng/ml) plus tamoxifen 1 μg/ml.

For each experiment the conditioned medium was collected and levels of LIF analysed. The remaining cells were solubilized in 0.1% SDS and the protein levels measured by Bio-Rad Protein Assay (Bio-Rad Glattbrugg, Switzerland), using bovine serum albumin (Sigma) as a standard. Each experiment was conducted in triplicate and repeated three times using cultures derived from different pools of fresh oviducts.

LIF ELISA

Aliquots (200 μl) of conditioned medium were used to analyse the presence of immunoreactive LIF using an ELISA kit (Quantikine™,
human LIF immunoassay; R&D Systems, Minneapolis, MN, USA; Arici et al., 1995; Cullian et al., 1996). According to the manufacturer’s specification, the minimal detectable concentration of LIF is 2 pg/ml and there is no significant cross-reactivity with other known cytokines. The interassay and intra-assay coefficients were 3.5 ± 0.4% and 4.4 ± 0.9% respectively. The concentrations of LIF were estimated using a standard curve, run under identical conditions. LIF concentrations were normalized to the total cell proteins and are presented as pg/mg protein.

**Expression of LIF mRNA in cultured oviduct epithelial cells/fibroblasts by reverse transcription–polymerase chain reaction (RT–PCR)**

Confluent monolayers of bovine oviduct epithelial/fibroblast cells in primary culture were washed twice with HBSS and the total RNA extracted by adding 500 µl of Ultraspec™ solution (Biotect, Veenendaal, The Netherlands). The samples were transferred into Eppendorf tubes and kept frozen at −70°C until analysed. Extraction of total RNA and analysis of mRNA expression was accomplished by using RT–PCR processing and polyacylamide gels stained with ethidium bromide as described previously (Van Eijk et al., 1996). Briefly, oligonucleotide primers used for amplification were based on human sequences in the databases, as follows: pLIF1 (5’-AACCTCATGACCAGATCG-3’) and pLIF2 (5’-TGACAGCCCAGCTTCTTCTT-3’), which yield a 431 bp product; actin-1 (5’-TGAACCCCAAGGCCAACCG-3’) and actin-2 (5’-TGAACCCCAAGGCCAACCG-3’) which yield a 396 bp product. The identity of the PCR products of these primer pairs was confirmed by cloning and sequencing.

**Statistical analysis**

Data are presented as a mean ± SEM or as a percentage of a control. Results were analysed by a paired t-test or analysis of variance using Statview program. P < 0.05 was considered to be statistically significant.

**Results**

**Cell characterization**

Immunostaining of epithelial cell preparations showed that >90% of the cells stained positively for cytokeratin with anti-cytokeratin AE1/AE3, whereas <10% of the cells stained positively for vimentin with anti-vimentin (Figure 1A). These findings indicate that the oviduct epithelial cell cultures used in this study consisted of ≥90% epithelial cells.

In preparations of fibroblasts incubated with anti-cytokeratin AE1/AE3, <5% of the cells stained positively for cytokeratin, whereas treatment with anti-vimentin positively stained >90% of the cells (Figure 1B). These observations provide evidence

**Figure 1.** Representative photomicrographs (×100, ×200) of cultured bovine oviduct epithelial cells, fibroblasts and mixed cell cultures (primary cultures) showing immunohistochemical labelling with monoclonal antibodies against fibroblast vimentin (anti-vimentin VIM 3B4). On each photomicrograph the positively stained fibroblasts are marked [a] and negatively stained cells marked [b]). The cells were reacted with primary antibodies and subsequently stained using a peroxidase–antiperoxidase method. Cell nuclei were counterstained with Harris haematoxylin. (A) Cultures of epithelial cells stained with anti-vimentin. (B) Cultures of fibroblasts stained with anti-vimentin. (C) Cultures of epithelial/fibroblast cells (mixed cultures) stained with anti-vimentin.
that the fibroblast cultures used in this study consisted of ~90% fibroblasts.

Immunohistochemical characterization of oviduct epithelial/fibroblast cells (mixed culture) showed that ~50 ± 5% cells stained positively for vimentin (anti-vimentin), indicating that in mixed cultures ~50% of cells were fibroblasts and 50% were epithelial cells (Figure 1C).

**Synthesis of LIF by bovine oviduct epithelial cells and fibroblasts**

Significant amounts of LIF were found in the conditioned medium collected after 4 days from monolayers of 90% epithelial cells, 90% fibroblasts and mixed cultures. The levels of LIF produced by the three different cell preparations did not vary significantly. In the supernatant of epithelial cell cultures, fibroblast cultures and mixed cultures of epithelial/fibroblast cells collected on day 4, the levels of LIF were: 272.8 ± 11.9, 347.7 ± 9.0 and 346.0 ± 22.0 pg/mg protein, respectively. These findings suggest that in the oviduct both the epithelial cells and the fibroblasts synthesize LIF and to a similar extent.

In mixed cell cultures the levels of LIF in the conditioned medium increased in a time-dependent fashion. As compared to the levels on day 1 (35.2 ± 3.7 pg/mg protein), the levels of LIF on days 5 and 7 were 121.5 ± 6.1 pg/mg protein (P < 0.05) and 215.7 ± 18.3 pg/mg protein (P < 0.05) respectively (Figure 2).

To confirm the ability of bovine oviduct cells to synthesize LIF we also assayed for the presence of its mRNA in mixed cultures. Amplification of cDNA primed with LIF or β-actin specific primers resulted in products of the expected size (431 and 398 base pairs respectively) after a single round of amplification for both genes in independent samples of mixed cultures from bovine oviduct. The identity of the 431 bp product using the LIF primers and the 398 bp product using the β-actin primers was confirmed by sequencing (data not shown). The detection of β-actin served as a positive control for RNA recovery and reverse transcription. No specific LIF or β-actin products were obtained in the absence of reverse transcriptase, or in water taken in a parallel control for RT–PCR. As shown in Figure 3, the PCR products visualized on polyacrylamide gels stained with ethidium bromide showed the expression of LIF mRNA (Figure 3).

LIF is involved in the implantation process and ovarian hormones and cytokines are known to influence the tissue remodelling process. Therefore we evaluated whether the ovarian hormones 17β-oestradiol and progesterone, TNFα, and insulin modulate the synthesis of LIF in the oviduct. Treatment of mixed cultures of the oviduct with 17β-oestradiol, but not progesterone, increased the levels of LIF in a concentration-dependent manner. The lowest concentration of 17β-oestradiol that significantly increased the levels of LIF was 10 ng/ml (Figure 4A). Moreover, the synthesis of LIF in epithelial cells, fibroblasts and mixed cultures in response to 17β-oestradiol, did not differ. At 100 ng/ml 17β-oestradiol increased the LIF levels in the conditioned medium by 61 ± 16.5% in epithelial cells, by 54 ± 7.5% in fibroblasts and by 66 ± 8.8% in mixed cultures compared to control (Figure 4B).

Since the synthesis of LIF by epithelial cells and fibroblasts was similar, we studied the effects of TNFα and insulin in mixed cultures. Treatment of mixed cultures with TNFα (100 ng/ml) significantly increased the levels of LIF in the conditioned medium. In contrast to TNFα, treatment with insulin (20 ng/ml) did not influence the synthesis of LIF (Figure 5). To confirm that the increase in LIF levels in response to 17β-oestradiol (50 ng/ml) was really due to LIF synthesis and not due to any non-specific reactions, we studied the effects of 17β-oestradiol (50 ng/ml) on LIF synthesis, by mixed cultures, in the presence and absence of cycloheximide (1 µM), an inhibitor of protein synthesis. As compared to untreated controls treatment of the cells with cycloheximide (1 µM) significantly reduced the exogenous levels of LIF. Moreover, in contrast to epithelial/fibroblast cells treated with 17β-oestradiol alone, the levels of LIF were dramatically reduced in cells treated with 17β-oestradiol (50 ng/ml) in the presence of cycloheximide (1 µM) (Figure 6). Additionally, we also evaluated the influence of calcium on LIF synthesis. Treatment of epithelial cells with EDTA (1 mM), a calcium chelator, did not influence the basal synthesis of LIF. However, in epithelial cells, EDTA (1 mM) significantly enhanced the stimulatory effects of 17β-oestradiol on LIF synthesis (Figure 6).

To identify whether the effects of 17β-oestradiol on LIF synthesis were receptor- or non-receptor-mediated, we studied the effects of 17β-oestradiol in the presence and absence of tamoxifen, an oestrogen receptor antagonist. Treatment of bovine oviduct mixed cell cultures with tamoxifen alone
induced synthesis of LIF in a concentration-dependent manner. Moreover, the effects of 17β-oestradiol on LIF synthesis were significantly enhanced in the presence of tamoxifen (Figure 7).

**Discussion**

The results of the present study demonstrate that bovine oviduct epithelial cells and fibroblasts synthesize LIF. Treatment of epithelial cells and fibroblasts with 17β-oestradiol, but not progesterone, stimulated LIF synthesis. The basal as well as 17β-oestradiol-stimulated synthesis of LIF in fibroblasts and epithelial cells did not vary. Similar to 17β-oestradiol, treatment of bovine oviduct mixed cell cultures with tamoxifen, an oestrogen receptor antagonist, also induced LIF synthesis and enhanced the effects of 17β-oestradiol on LIF synthesis, suggesting that the effects were non-receptor-mediated. Furthermore, treatment of mixed cultures with TNFα, but not insulin, stimulated the synthesis of LIF. Taken together our findings provide evidence that 17β-oestradiol, as well as TNFα, stimulates the synthesis of LIF by oviduct epithelial cells and fibroblasts and this may regulate the development of the embryo for the implantation process in an autocrine/paracrine fashion.

The observation that the levels of LIF in the conditioned medium of bovine oviduct mixed cell cultures increased in a time-dependent fashion suggests that LIF is continuously synthesized within the oviduct under basal conditions. This notion is further supported by our observation that mixed cultures expressed the mRNA for LIF and by the finding that cycloheximide, an inhibitor of protein synthesis, dramatically suppressed the production/concentrations of LIF in the conditioned medium.

Within the oviduct the cell–cell interaction between the epithelial cells and the fibroblasts plays an important role in maintaining the morphological structure and physiological function of the oviduct. Moreover, in the female reproductive tract, several factors generated by the fibroblasts have been shown to influence epithelial cell function in an autocrine/paracrine fashion (Osteen et al., 1994). To investigate whether the production of LIF by bovine oviduct epithelial cells is influenced by factors generated by fibroblasts, we studied and compared the synthesis of LIF in epithelial cell cultures, cultures of fibroblasts and in mixed cultures. Basal synthesis of LIF was observed in both the epithelial cells and fibroblasts, as well as in mixed cultures, and the levels of LIF produced did not differ. This finding provides evidence that the fibroblasts are as efficient as epithelial cells in producing LIF and that basal synthesis of LIF is not influenced by epithelial–fibroblast cell interaction.

Treatment of epithelial cell cultures and fibroblast cell cultures as well as mixed cultures from the bovine oviduct with 17β-oestradiol, but not progesterone, stimulated LIF synthesis in a concentration-dependent manner, suggesting that 17β-oestradiol stimulates LIF synthesis by both the oviduct epithelial cells and fibroblasts. Since LIF is essential for implantation (Stewart et al., 1992), it is feasible that 17β-oestradiol together with other cytokines stimulates the physiological release of LIF within the oviduct, which in turn influences the development of the embryo and prepares it for implantation.

In contrast to our findings, an earlier study by Keltz et al. (1996) failed to show any increase in immunoreactive LIF levels in the conditioned medium of human oviduct epithelial cells treated with 17β-oestradiol. The reasons for the different observations in the two studies may be due to species differences (bovine, human) or due to the conditions under which the experiments were conducted. In the present study, to mimic a more physiological environment, all the treatments were done in the presence of 1% FCS (charcoal-stripped), whereas Keltz et al. (1996) treated the cells in the absence of serum. Because serum contains a battery of growth factors and is known to induce LIF synthesis in uterine epithelial cells (Keltz et al., 1996), it is feasible that 17β-oestradiol requires some essential factors present in the serum to induce its effects. Alternatively, the cells are more active in presence of serum and respond differently to 17β-oestradiol compared to cells grown in serum-free medium. However, further studies will be needed to confirm these findings.

The finding that 17β-oestradiol, but not progesterone and

![Figure 3](image-url). Expression of leukaemia inhibitory factor (LIF) and β-actin in bovine oviduct mixed cultures (primary cell culture) by reverse transcriptase–polymerase chain reaction (RT-PCR). The products were visualized on polyacrylamide gels stained with ethidium bromide. Two independent samples (1 and 2) showed the expression of LIF mRNA. A 100 bp ladder is included as a marker of fragmentation. The markers shown in the upper panel are for 400 and 500 bp, and in the lower panel are for 300 and 400 bp.
Figure 4. (A) Bar graph showing the concentration–response curve for the effects of 17β-oestradiol (1–100 ng/ml) on the synthesis of leukaemia inhibitory factor (LIF) by confluent monolayers of bovine oviduct mixed cell cultures (first passage) grown in Dulbecco’s modified Eagle’s medium (DMEM)/Ham’s F12 medium containing 1% fetal calf serum (FCS; charcoal-stripped). Cells were cultured for 4 days in the presence or absence (control) of 17β-oestradiol and the levels of LIF assayed in triplicate in the supernatant using LIF enzyme-linked immunosorbent assay (ELISA). Data (mean ± SEM; n = 3) are from a representative experiment and values were normalized to total protein concentration. Similar results were obtained in three independent experiments. The amount of LIF synthesized is expressed as pg/mg protein. *P < 0.05 versus control (1% FCS).

(B) Bar graph comparing the concentration–response curve for the effects of 17β-oestradiol (1–100 ng/ml) on the synthesis of LIF by confluent monolayers of cultured bovine oviduct epithelial cells (epc, primary cell culture) and fibroblasts (fibro, first passage). Cells were grown in DMEM/Ham’s F12 medium containing 1% FCS (charcoal-stripped). Cells were cultured for 4 days in the presence or absence (control) of 17β-oestradiol and the levels of LIF assayed in triplicate in the supernatant using LIF enzyme-linked immunosorbent assay (ELISA). Data (mean ± SEM; n = 3) were normalized to total protein concentration and expressed as percentage of control (C). The increases of the levels of LIF in the two different cell types (epithelial cells versus fibroblasts) in response to 1, 10 and 100 ng/ml 17β-oestradiol (*P < 0.05) were similar and did not vary.

Figure 5. Bar graph showing the effects of tumour necrosis factor-α (TNFα), progesterone and insulin on leukaemia inhibitory factor (LIF) synthesis by bovine oviduct mixed cell cultures (first passage). TNFα (100 ng/ml) significantly (*P < 0.01) increased the synthesis of LIF by confluent monolayers of mixed cell cultures (first passage) treated for 4 days in Dulbecco’s modified Eagle’s medium/Ham’s F12 medium with 1% fetal calf serum (charcoal-stripped), whereas insulin 20 ng/ml (I) and progesterone 50 ng/ml (P) had no effect. Data (mean ± SEM) were normalized to total protein concentration and are expressed as percentage of control (C). All experiments were conducted in triplicates. Results represent mean ± SEM of three separate cultures from fresh oviducts (n = 3).

Figure 6. Bar graph showing the modulatory effects of cycloheximide 1 µM (Cy) and EDTA 0.1 mM on 17β-oestradiol 50 ng/ml (E)-induced synthesis of leukaemia inhibitory factor (LIF) by confluent monolayers of bovine oviduct mixed cell cultures (primary cell culture) grown in Dulbecco’s modified Eagle’s medium/Ham’s F12 medium containing 1% fetal calf serum (charcoal-stripped). Levels of LIF in the conditioned medium collected after 4 days of treatment were measured by LIF enzyme-linked immunosorbent assay in triplicate. Data (mean ± SEM; n = 3) were normalized to total protein concentration and expressed as percentage of control (C). E as well as EDTA+E significantly increased the synthesis of LIF (*P < 0.01 versus control; †P < 0.01 versus 17β-oestradiol 50 ng/ml). Cycloheximide 1 µM (Cy) inhibited basal and 50 ng/ml 17β-oestradiol-induced synthesis of LIF (Cy+E) (*P < 0.01 versus control).
insulin, stimulated the levels of LIF in the conditioned medium suggests that the production of LIF is differentially regulated by hormones. Moreover, it also provides evidence that the observed increases in LIF levels were real and not due to the leakage or non-specific breakdown of the protein. This contention is further supported by our observation that both the basal and the 17β-oestradiol-induced synthesis of LIF was blocked in presence of the protein synthesis inhibitor cycloheximide. The expression and synthesis of LIF in the endometrium and placenta has been documented (Bhatt et al., 1991; Kojima et al., 1994; Arici et al., 1995; Cullinan et al., 1996). In the endometrium the modulation of LIF expression seems to be differentially regulated by hormones. Additionally, abundant mRNA for LIF has been shown to be expressed in the mid and late luteal phase (Arici et al., 1995), suggesting that it may be under progesterone control. Based on our finding that 17β-oestradiol had a stimulatory effect on LIF synthesis, whereas progesterone had no effect, it is feasible that LIF synthesis is differentially regulated in the uterus and the oviduct, which play different roles in the implantation process. Moreover, since the present study was performed in bovine oviduct, these findings may not apply to humans.

To investigate whether the effects of 17β-oestradiol were receptor-operated, we studied its effects in the presence and absence of tamoxifen, a non-steroidal oestrogen receptor antagonist (Jordan et al., 1980). The observation that tamoxifen induced LIF synthesis in bovine oviduct cells and enhanced the effects of 17β-oestradiol on LIF synthesis suggests that the effects of 17β-oestradiol are non-receptor-mediated. The fact that the effects of 17β-oestradiol on LIF synthesis were enhanced in an additive fashion suggests that tamoxifen and 17β-oestradiol induce LIF synthesis via different mechanisms.

Indeed, tamoxifen, but not 17β-oestradiol, is known to increase TGFβ levels (Grainger et al., 1995) and TGFβ has been shown to induce LIF synthesis (Keltz et al., 1996).

The observation that the basal synthesis of LIF was not influenced by EDTA, whereas 17β-oestradiol-induced LIF synthesis was enhanced in the presence of EDTA, suggests that the cascade of enzymes responsible for the synthesis of LIF is calcium independent. Alternatively, it is feasible that the increase in LIF production in the presence of EDTA is due to inactivation or decreased activity of calcium-dependent proteolytic enzymes that degrade LIF. However, further studies will be required to define the mechanism of action.

What may be the physiological relevance for the synthesis of LIF within the oviduct? The oviduct not only provides a conduit for the transport of the spermatozoa and the oocyte, but more importantly creates the microenvironment where fertilization and the early stages of embryo development take place. Successful implantation of a developing embryo within the uterus is a time-dependent process. Therefore it is feasible that the microenvironment of the oviduct is essential for early embryo development and to prime or condition the embryo for implantation in the uterus. Regarding plausible mechanisms, it is likely that LIF promotes the expression of certain anchoring cell-surface proteins essential for binding. Indeed, LIF has been shown to play a pivotal role in shifting trophoblast differentiation towards a tropho-uteronectin-secreting anchoring phenotype by 4–8-cell human embryos, which facilitates their binding to the endometrial integrins at the time of trophoblast penetration (Turpeenniemi-Hujanen et al., 1995; Nachtigall et al., 1996). Moreover, lack of synthesis of LIF within the oviduct may also result in an embryo deficient of anchoring proteins and incapable of implanting and therefore resulting in abortion. Indeed, in mice lacking LIF implantation does not occur (Stewart et al., 1992), whereas embryos pretreated with LIF in vitro have a greater rate of implantation than those not primed with LIF (Fry et al., 1992).

Although LIF generated physiologically is important for the successful implantation of the embryo, an abnormal production of LIF in the oviduct may participate in the implantation of the embryos within the oviduct and may be associated with the pathology of ectopic pregnancy. In a recent study by Keltz et al. (1996) the expression of LIF was dramatically increased in the oviducts obtained from patients with ectopic pregnancy. In a recent study by Keltz et al. (1996) the expression of LIF was dramatically increased in the oviducts obtained from patients with ectopic pregnancy. Moreover, the incidence of ectopic pregnancy increases in the presence of endometriosis or other inflammatory processes. Hence, it is feasible that an additive/synergistic effect of 17β-oestradiol with cytokines, e.g. TNFα released in conjunction with an inflammatory process, may contribute to an increased release of LIF under pathological conditions. Moreover, the pathological increase in LIF may subsequently lead to implantation of the embryo within the oviduct, resulting in ectopic pregnancy.

In conclusion, we provide the first evidence that 17β-oestradiol stimulates the synthesis of LIF from bovine oviduct epithelial cells as well as fibroblasts and that these effects are not mimicked by progesterone and insulin. Moreover, the stimulatory effects of 17β-oestradiol were receptor independent. Finally, the findings of this study support the concept that
the oviduct not only generates the microenvironment for the fertilization process, but also synthesizes growth factors and cytokines under the control of sex steroids, which may subsequently determine/regulate the development of preimplantation embryos.

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References


