Characterizing LIF, LIFR, and gp130 Expression in Patient Tissue and 3D Endometrial Organoids

Manavi Vajhallya, Elizabeth E. Marr, Juan S. Gnecco

Advisor: Juan S. Gnecco

Department of Biomedical Engineering, Tufts University

Laidlaw Scholars Program

ABSTRACT

Leukemia inhibitory factor (LIF) is a cytokine critical for endometrial receptivity and embryo implantation, signaling through its receptor complex comprised of LIFR and gp130. While LIF signaling has been extensively studied in mouse models, its expression and regulation in the human endometrium remain poorly understood. In this study, we analyzed LIF, LIFR, and gp130 expression in patient endometrial tissue across different menstrual cycle phases using immunohistochemistry (IHC), and in patient-derived endometrial epithelial organoids (EEOs) via immunofluorescence. First, estrogen receptor staining was used to confirm protocol reliability. For the selected tissue samples, LIF, LIFR, and gp130 were not detected, suggesting biological variability and technical limitations may hinder detection *in vivo*. In contrast, 3-dimensional (3D) organoid models exhibited robust LIF expression in multiple samples and detectable LIFR in at least one, while gp130 remained undetected. These findings indicate that organoids might provide a more sensitive and physiologically relevant platform for studying human endometrial cytokine signaling. Our results highlight the potential of 3D organoid systems to investigate temporal regulation of LIF signaling and its role in fertility and endometriosis, offering a promising avenue for future research into reproductive health.

BACKGROUND

The endometrium is the inner lining of the uterus that plays a pivotal role in pregnancy and the menstrual cycle. The menstrual cycle is predominantly influenced by

two hormones, estrogen and progesterone, and consists of four phases. In the proliferative phase of the cycle, estrogen stimulates growth and development of endometrial stroma and glands. This is followed by ovulation, which occurs right after a peak in estrogen levels, and the secretory phase, in which progesterone production helps establish and maintain pregnancy if it occurs. If pregnancy does not occur, the endometrium is shed in the final phase of the cycle, menses (Thiyagarajan et al., 2025).

Leukemia inhibitory factor (LIF) is a cytokine in the Interleukin 6 (IL6) cytokine family with both pro-inflammatory and anti-inflammatory properties. It is important in fertility and known to be regulated when the endometrium is receptive during the menstrual cycle. During the menstrual cycle, LIF expression has been found to be downregulated during the proliferative phase, and increase during ovulation, remaining relatively high during the secretory phase (Chen et al., 1995; Vogiagis et al., 1996). Deficiency in LIF has been associated with infertility due to the failure of embryo attachment (Namiki et al., 2023; Stewart et al., 1992). Studies of endometrial biopsy samples have shown that most infertile women showed no increase of LIF production during the secretory phase, with varying LIF levels during the proliferative phase (Hambartsoumian, 1998; Lass et al., 2001).

The LIF receptor complex is made up of two heterodimers: LIF receptor- β (LIFR) and the glycoprotein 130 (gp130). The binding of LIF triggers the dimerization of LIFR and gp130 and induces the phosphorylation of the STAT3 pathway (Xie et al., 2007). In mouse models, LIFR is found in both luminal and glandular epithelium before embryo implantation, while gp130 is found primarily in the glandular epithelium (Ni et al., 2002; Sun et al., 2013; Yang et al., 1995). Gene knockout studies with mice have shown that

mice with LIF knock out were found to be infertile, indicating that LIF-mediated signaling plays an important role in infertility (Stewart et al., 1992). A recent study with LIFR deficiency in uterine epithelial cells has shown greater incidences of embryo implantation failure compared to LIFR deficiency in uterine stromal cells, which showed no obvious phenotypes (Cheng et al., 2017). In another study for gp130, uterine-specific gene knockout females presented infertility due to an increased response to estrogen and a decreased response to progesterone (Namiki et al., 2023; Sun et al., 2013).

Organoids are multicellular 3D structures that mimic the architecture and function of *in vivo* tissue and can be used to better understand uterine biology. Endometrial epithelial organoids (EEOs) are derived from endometrial glands isolated from a patient's endometrium and expanded. Various hormones and inhibitors in the organoid media simulate *in vivo* conditions, and hormones such as estrogen and progesterone encourage EEO proliferation and differentiation (Marr et al., 2025). EEOs can serve as a model system to better understand LIF signaling in the human endometrium. Current knowledge about the LIF signaling pathway and the LIFR and gp130 receptor complex is primarily from mouse models. However, the mouse reproductive system is significantly different from the human reproductive system. The mouse does not menstruate, and the human reproductive organs (open reproductive system) differ anatomically from the mouse reproductive organs (closed reproductive system) (Burns et al., 2021). Thus, LIF signaling and its relationship with LIFR and gp130 needs to be better understood in human models to address infertility in humans and further develop therapies.

MATERIALS AND METHODS

Endometrial biopsy sample processing and organoid generation

Endometrial biopsies were isolated via pipelle biopsy from female patients of reproductive page (18 – 45 years) undergoing minimally invasive surgeries for endometriotic lesion removal or other gynecological procedures. Patients were consented by Newton Wellesley Hospital and excluded if they had received hormone-based treatment 3 months prior to the procedure. Endometrial biopsies were transferred via courier to Tufts University, where they were further processed for histology or primary cell isolations and organoid development. Briefly, to generate histological samples, 3mm sized tissue chunks were fixed in 4% paraformaldehyde at 4°C and transferred to the Tufts Histology Processing Core to be paraffin embedded. For endometrial epithelial organoid (EEO) generation, endometrial tissue was washed, minced, and then enzymatically digested at 37°C. Stromal cells were separated from endometrial glands via cell filtering, then cell populations were expanded separately in 2D or 3D culture via Matrigel embedding, respectively.

Organoid Seeding and Maintenance

EEOs across various donors (NWH004, NWH008, NWH014) were resuspended in Matrigel, then plated as domes in 5 μL (in 96 well tissue culture plates) or 50 μL volumes (in 24 well tissue culture plates). EEOs were supplemented with EEO media (ABM, ITS, rhEGF, rhNoggin, A8301, Nicotinamide, N-Acetyl-L-Cysteine, Estradiol, rhFGF-10, and rhRspondin-1 in DMEM/F12) and media was refreshed every 3 days prior to takedown for immunofluorescent staining.

3D Immunofluorescence Staining for Organoids

Organoids were fixed in 4% paraformaldehyde for 30 minutes at room temperature, then washed in phosphate buffered saline (PBS). Following fixation, organoids were permeabilized in permeabilization buffer (0.5% Triton X-100 in PBS) for 30 minutes and blocked in 5% normal goat serum in 1% BSA overnight. Primary antibody staining was performed overnight for LIF (LIF Polyclonal Antibody, Invitrogen PA5-79600), LIFR (LIFR Polyclonal antibody, proteintech 22779-1-AP), and gp130 (Human gp130 Antibody, R&D Systems MAB628). Between blocking and antibody staining steps, organoids were washed three times in 1% bovine serum albumin in PBS. Finally, organoids were incubated in secondary antibody overnight and imaged on a ZEISS LSM 900 Confocal microscope (ZEISS, Oberkochen, Germany).

Immunohistochemistry

Paraffin-embedded tissue samples were sectioned (7μm) and deparaffinized and hydrated in xylene, varying concentrations of ethanol (100% two times, 95% two times, 70% once, and 50% once), and deionized water. Antigen retrieval was performed in a pressure cooker in 10 mM sodium citrate buffer (pH = 6.0) for 25 minutes, then allowed to cool to room temperature. Tissues were incubated in 3% hydrogen peroxide in methanol for 20 minutes and blocked in 5% normal goat serum for 30 minutes, with washes of PBS in between. Primary antibody staining was performed with LIF (LIF Polyclonal Antibody, Invitrogen PA5-79600 1:500), LIFR (LIFR Polyclonal antibody, proteintech 22779-1-AP 1:50), and gp130 (Human gp130 Antibody, R&D Systems MAB628 1:50) overnight. After incubation in secondary antibody (Goat Anti-Rabbit IgG Antibody, Biotinylated, Vector Laboratories ba-1000-1.5 and Goat Anti-Mouse IgG

Antibody, Biotinylated, Vector Laboratories ba-9200-1.5) for 30 minutes, streptavidin peroxidase (VECTASTAIN® Elite® ABC-HRP Kit, Peroxidase, Vector Laboratories PK-6100) was applied for 30 minutes. Washes of PBS-T were performed in between these steps. Positive antibody staining was visualized by 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Pierce™ DAB Substrate Kit, ThermoScientific, 34002) and counterstained with hematoxylin. Tissue samples were dehydrated and mounted with Fluoromount G. Samples were imaged on a Keyence 710 microscope (Keyence, Osaka, Japan).

RESULTS AND DISCUSSION

Tissue Samples

Patient tissue was obtained from endometrial biopsy or hysterectomy samples. Twelve viable samples were processed and evaluated via hematoxylin and eosin (H&E) staining. The menstrual cycle phase of each sample was determined based on glandular morphology, and the presence of endometriosis was determined via pathology report. As LIF is typically upregulated during the mid- to late-secretory phase of the menstrual cycle, samples A, B, C, and D were selected for analysis of LIF expression. Sample E, collected during the proliferative phase, was used to test expression of estrogen receptor (ESR).

Table 1. Characteristics of patient samples, including pathology, age, and menstrual cycle phase.

Donor ID	Pathology	Patient Age	Menstrual Cycle Phase
Sample A	Endometriosis	29	Mid secretory

Sample B	Endometriosis	39	Late secretory
Sample C	Control	39	Mid secretory
Sample D	Control	32	Early secretory
Sample E	Control	29	Proliferative

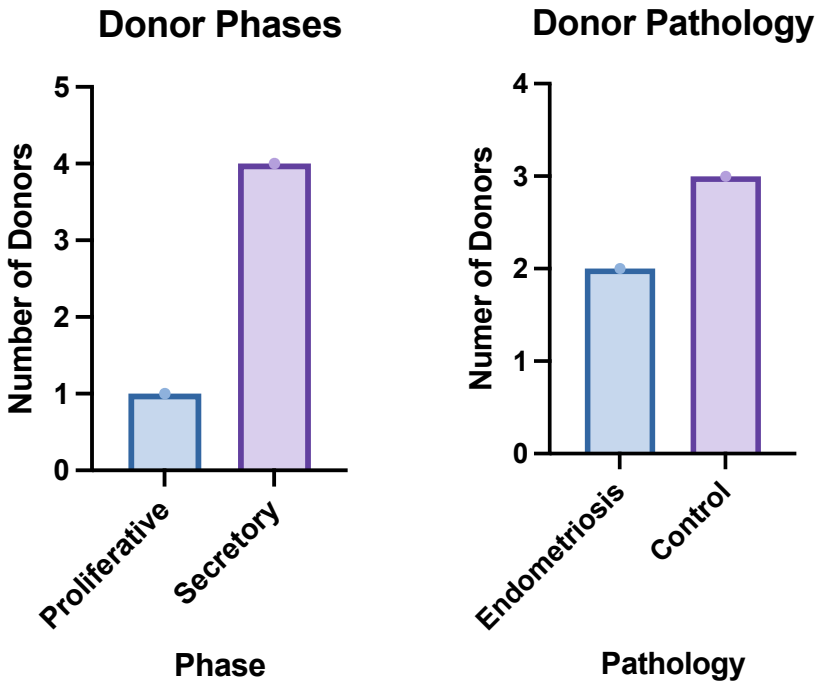
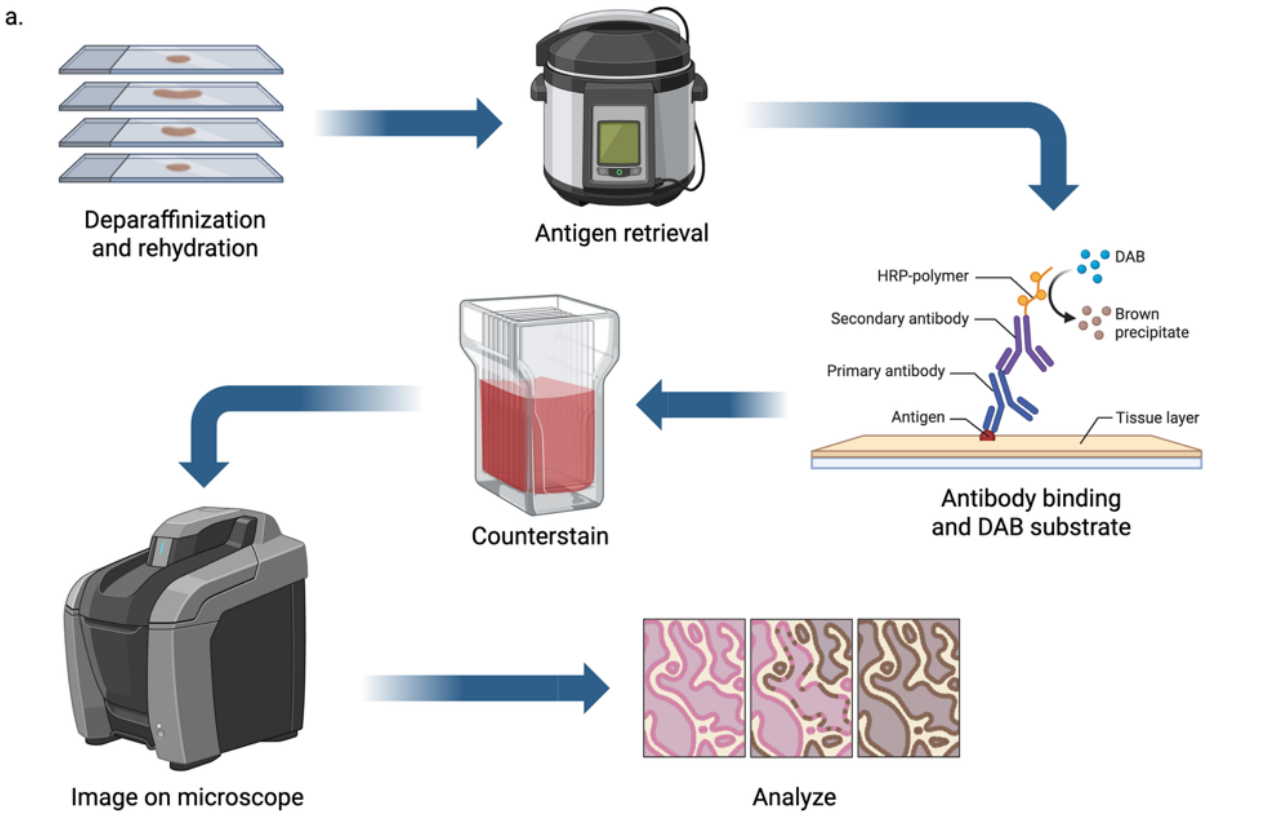


Figure 1. Distribution of donor samples according to menstrual cycle phase (left) and pathology (endometriosis vs. control) (right).

The IHC protocol was validated by performing ESR staining on Samples D and E, as ESR expression is expected across all menstrual cycle phases. Antigen retrieval was optimized by comparing two buffers: 10 mM sodium citrate (pH 6.0) and 10mM Tris (pH 8.5). Sodium citrate provided the most consistent results. Retrieval time was tested at 15, 20, and 30 minutes of incubation in an Instant Pot (Kearns et al., 2023), with optimal staining observed at 20–25 minutes. Additional parameters, including incubation time with the secondary antibody and hematoxylin counterstaining, were also refined. Following confirmation of positive ESR staining, the optimized IHC protocol was applied to detect LIF, LIFR, and gp130.



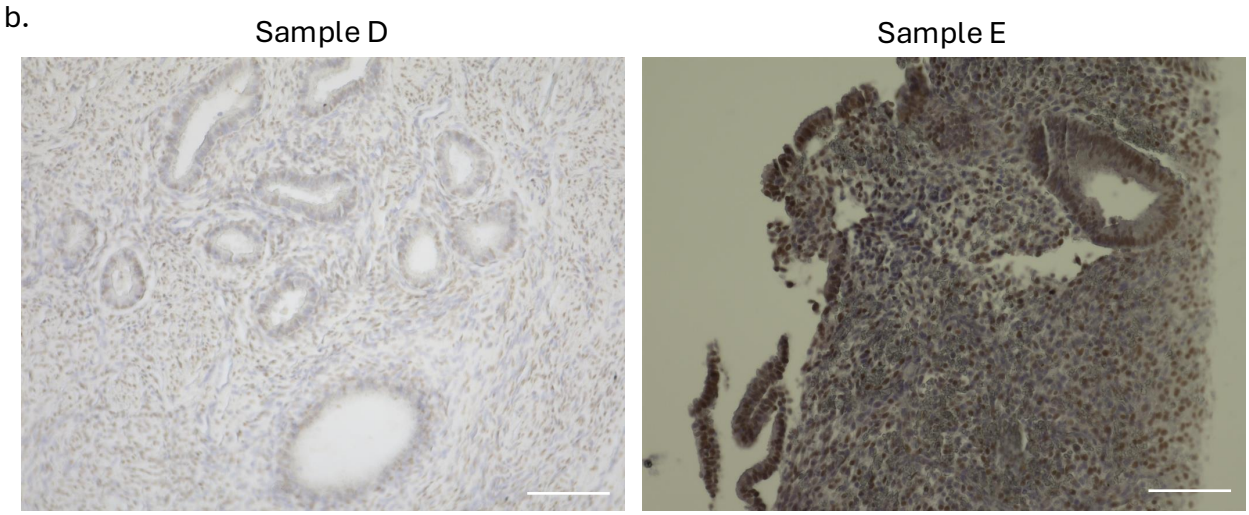
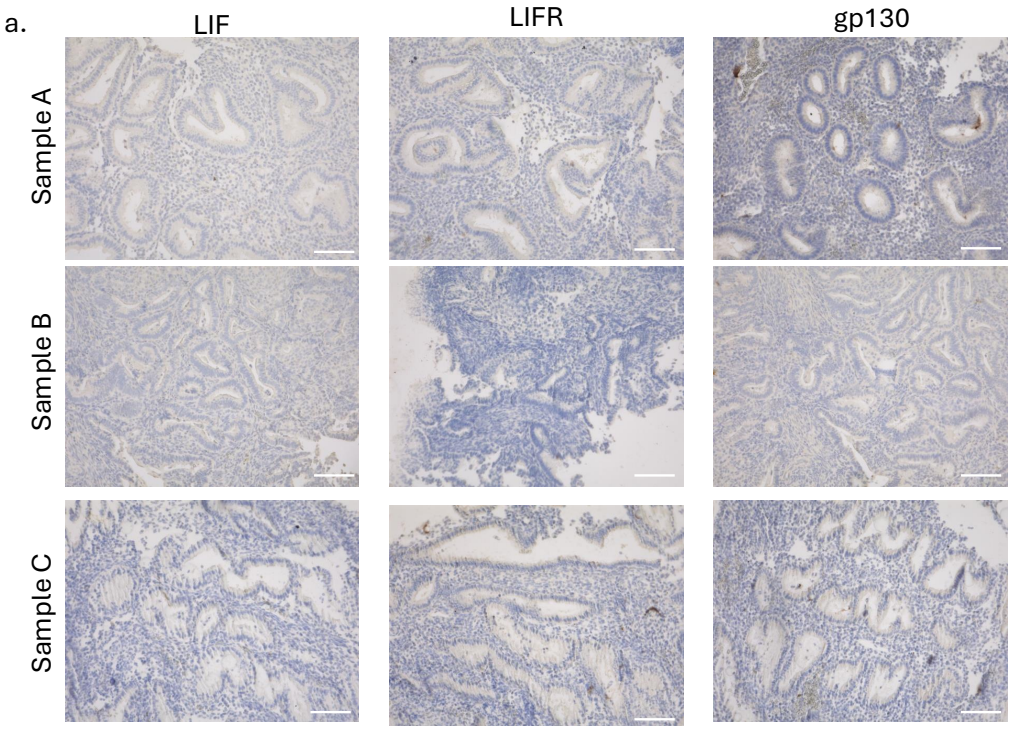


Figure 2. (a) Flowchart of the optimized IHC protocol. (b) ESR staining in Sample D (left) and Sample E (right). ESR is stained in brown and hematoxylin is light purple. All scale bars are 100 μm.

IHC was performed on Samples A, B, and C to assess expression of LIF, LIFR, and gp130, with ESR staining included as a positive control in Sample C. As these samples were collected during the secretory phase, positive LIF staining was

anticipated. However, neither LIF, LIFR, gp130, nor ESR expression was detected. To confirm the reliability of the experiment, it was repeated using Sample D. In this case, ESR expression was successfully detected, verifying the validity of the protocol, but LIF, LIFR, and gp130 remained negative. These findings suggest that while the IHC procedure was technically successful, the absence of detectable LIF/LIFR/gp130 expression could reflect biological variability tied to menstrual cycle phase. Since these markers appear only during a brief stage of the cycle, obtaining patient tissue at the precise time of expression can be challenging. Alternatively, technical factors such as too much antibody dilution or insufficient antigen retrieval conditions for these specific proteins may have contributed to the lack of staining.



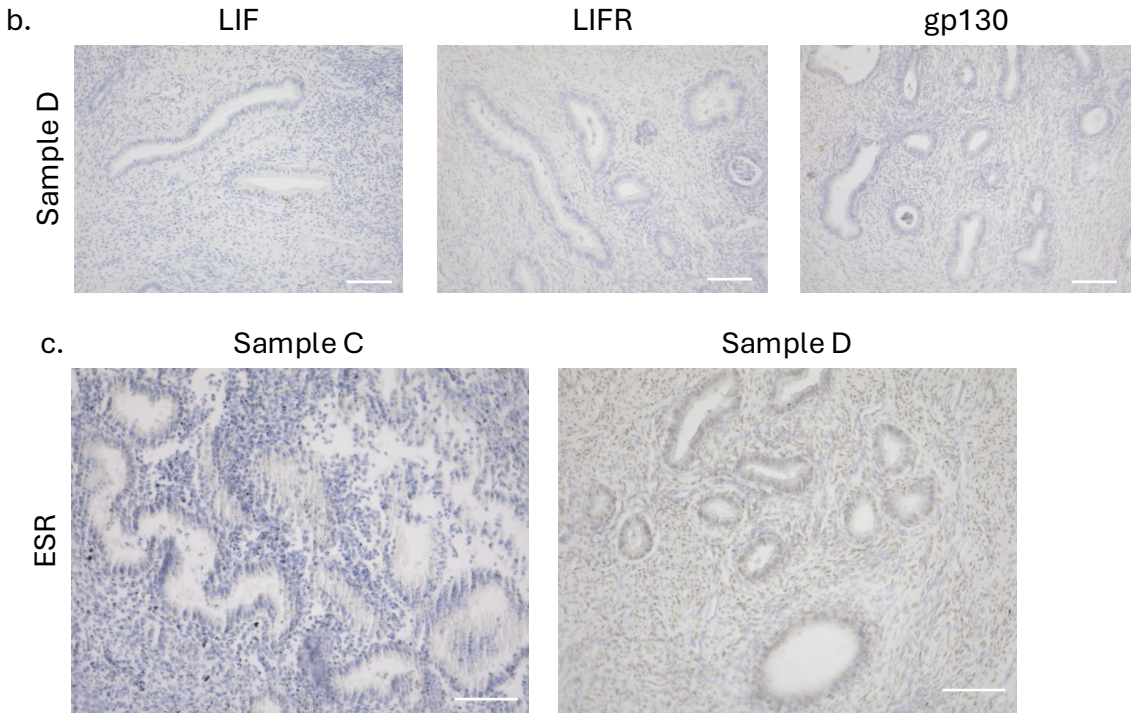


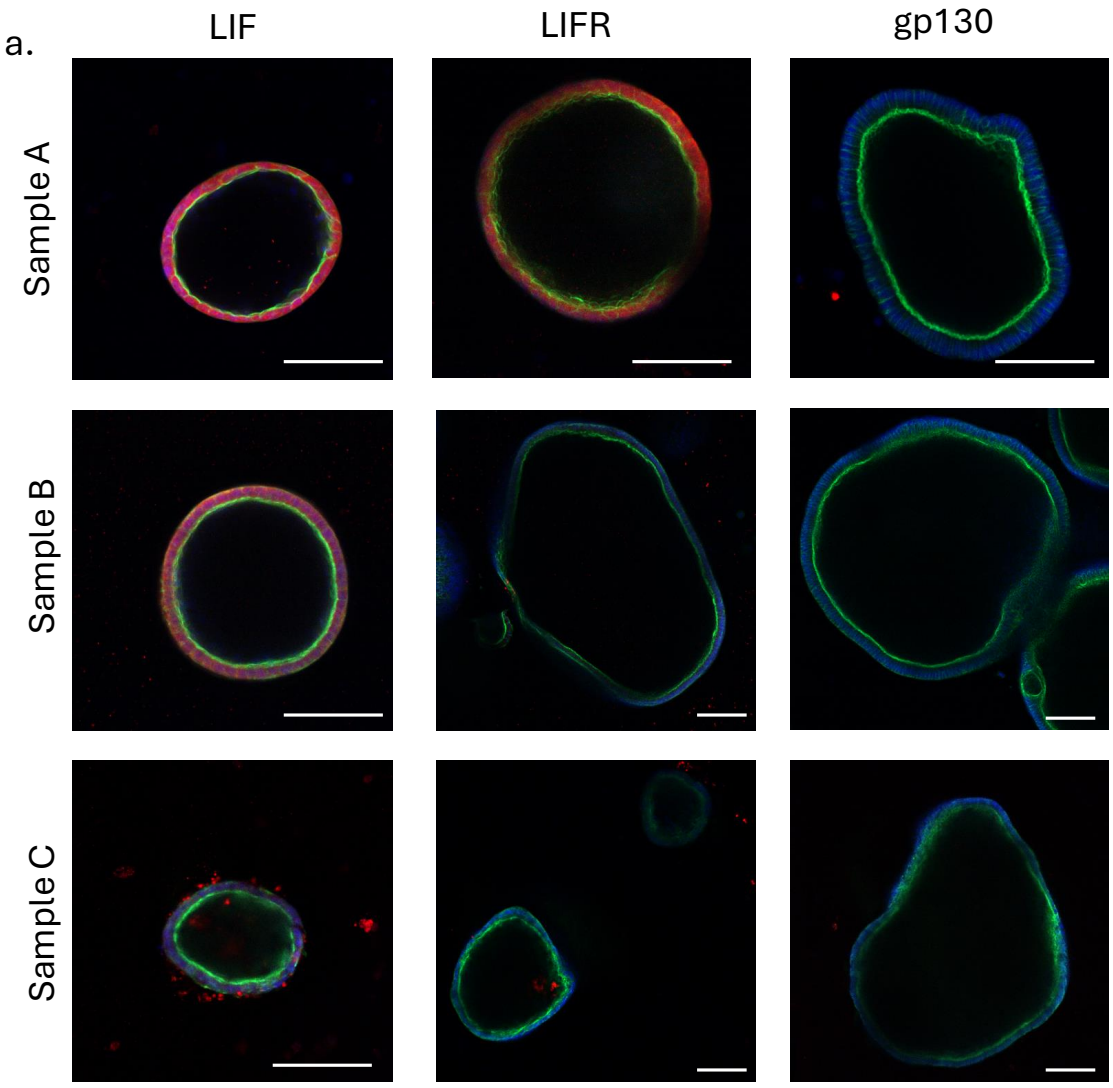
Figure 3. (a) LIF, LIFR, and gp130 staining in Samples A, B, and C. (b) LIF, LIFR, and gp130 staining in Sample D. (c) ESR staining in Sample C and Sample D. Brown indicates positive staining for ESR/LIF/LIFR/gp130 and light purple is hematoxylin staining. All scale bars are 100μm.

Further experiments are required to show expression of LIF/LIFR/gp130 in patient tissue using IHC. These experiments can optimize the protocol by testing different antibody dilutions and refining antigen retrieval conditions. Additionally, having many patient tissue samples across varying parts of the menstrual cycle could contribute to a more comprehensive understanding of marker expression.

Organoids

Organoids were seeded from Samples A, B, and C and stained for LIF, LIFR, and gp130. Positive LIF expression was observed in Samples A and B (10 days of culture), with considerably lower expression in Sample C (70 days of culture). The reduced signal in Sample C may reflect the older age of this organoid, suggesting that LIF expression declines over time in culture. Sample A also showed detectable LIFR

expression, whereas Samples B and C did not. No gp130 expression was observed in any of the samples. A secondary only control was performed for both Alexa Fluor 594 and Alexa Fluor 555 to account for species-specific secondary antibodies (gp130 antibody raised in mouse; LIF and LIFR antibodies raised in rabbit). These controls confirmed that the observed staining was not due to background fluorescence.



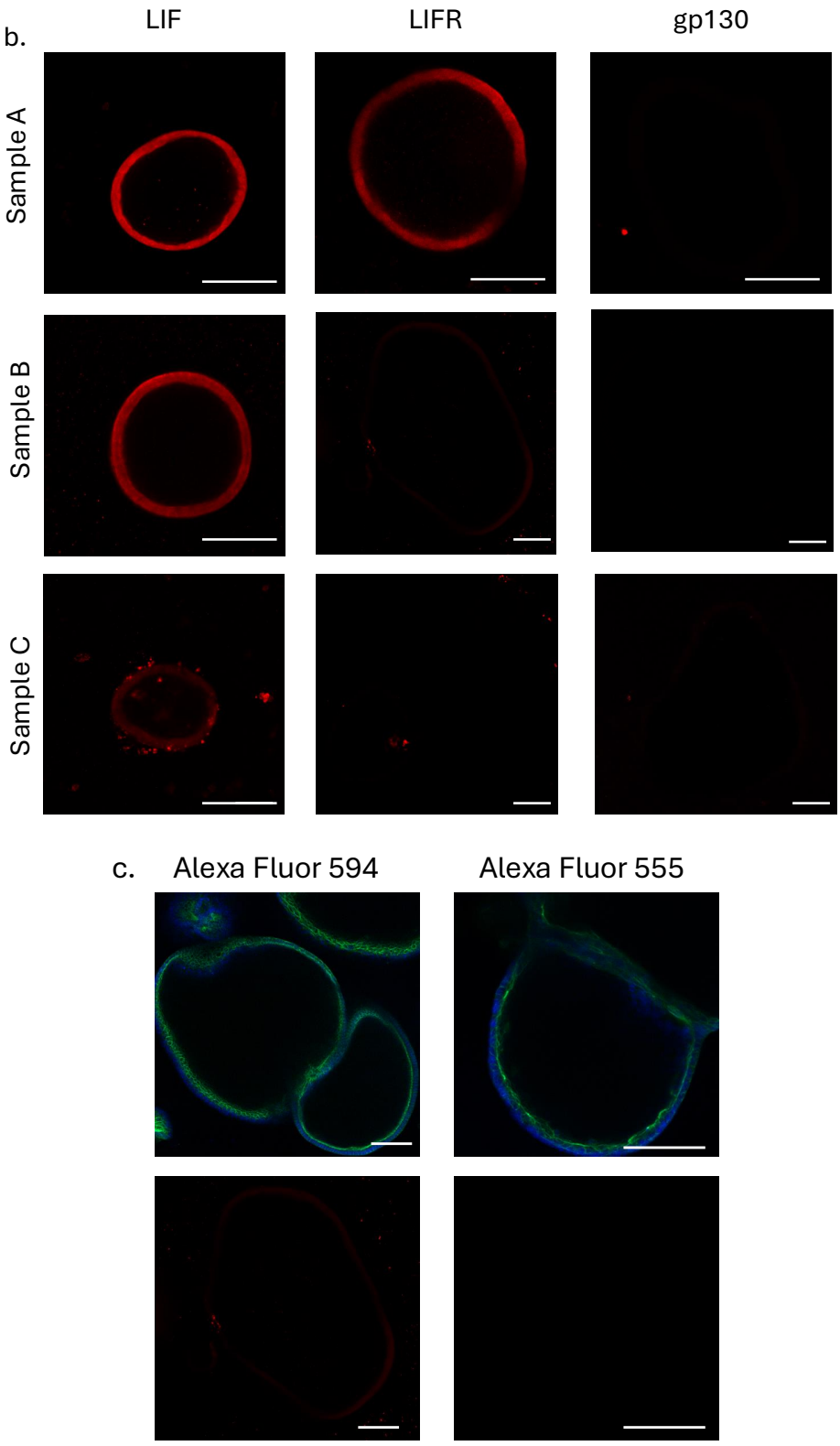


Figure 4. (a) Immunofluorescence staining of organoids from Samples A, B, and C. Green = actin; blue = DAPI; red = LIF/LIFR/gp130. (b) Red channel only for Samples A, B, and C. (c) Secondary only control for Sample B, shown with all channels (top) and red channel only (bottom). All scale bars are 100μm.

These findings demonstrate that 3D endometrial organoid models exhibit expression of LIF and even LIFR to some extent. The lack of gp130 detection suggests either restricted expression in this context or technical limitations. The 3D architecture of organoids may contribute to their ability to support cytokine signaling in a way that better reflects in vivo endometrial tissue. In addition, organoids preserve epithelial polarity, maintain diverse cell populations, and remain hormonally responsive, all of which likely promote LIF and LIFR expression compared to 2D models. These results indicate that while LIF and LIFR expression is difficult to detect in patient tissue using standard IHC, 3D organoid models provide a more sensitive platform for studying dynamic LIF expression. Consequently, organoids may serve as a valuable tool for investigating endometrial cytokine signaling and the temporal regulation of these markers across the menstrual cycle.

CONCLUSIONS AND FUTURE DIRECTIONS

This study demonstrates that LIF and LIFR expression in human endometrium is challenging to detect in patient tissue via standard immunohistochemistry, likely due to biological variability across menstrual cycle phases and potential technical limitations. In contrast, endometrial epithelial organoids effectively capture aspects of *in vivo* tissue architecture and hormonal responsiveness, allowing for detectable LIF and LIFR expression, highlighting their potential as a model system for studying cytokine signaling in the human endometrium.

Future studies should focus on expanding the number of patient-derived samples across all menstrual cycle phases to better capture the temporal dynamics of LIF and LIFR expression in patient tissue samples. A better understanding of LIF and its interactions with LIFR and gp130 could contribute to a better understanding of infertility in women. This knowledge could contribute to a better understanding of endometriosis, a disease in which the endometrium grows in non-uterine tissue, often resulting in infertility. Endometriosis has been associated with reduced LIF production (Zutautas et al., 2023). Thus, advancing the understanding of LIF signaling in the endometrium is vital for understanding further endometrial dysfunction, such as infertility and endometriosis.

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