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ORIGINAL RESEARCH ARTICLE

Cyclic activity of signal transduction pathways in fimbrial epithelium of the human fallopian tube

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Abstract

Introduction: The local environment of the fallopian tube represents the optimal conditions for reproductive processes. To maintain tissue homeostasis, signal transduction pathways are thought to play a pivotal role. Enhancing our understanding of functional signal transduction pathway activity is important to be able to clarify the role of aberrant signal transduction pathway activity leading to female subfertility and other tubal diseases. Therefore, in this study we investigate the influence of the hormonal cycle on the activity of key signal transduction pathways in the fimbrial epithelium of morphologically normal fallopian tubes.

Material and methods: We included healthy pre- ($n = 17$) and postmenopausal ($n = 8$) patients who had surgical interventions for benign gynecologic conditions. Histologic sections of the fallopian tubes were reviewed by two pathologists and, for the premenopausal patients, hormone serum levels and sections of the endometrium were examined to determine the hormonal phase (early follicular [$n = 4$], late follicular [$n = 3$], early luteal [$n = 5$], late luteal [$n = 5$]). After laser capture microdissection, total mRNA was extracted from the fimbrial epithelium and real-time quantitative reverse transcription-PCR was performed to determine functional signal transduction pathway activity of the androgen receptor (AR), estrogen receptor (ER), phosphoinositide-3-kinase (PI3K), Hedgehog (HH), transforming growth factor-beta (TGF-β) and canonical wingless-type MMTV integration site (Wnt) pathways.

Results: The early luteal phase demonstrated high AR and ER pathway activity in comparison with the late luteal phase ($p = 0.016$ and $p = 0.032$, respectively) and low PI3K activity compared with the late follicular phase ($p = 0.036$), whereas the late luteal phase showed low activity of HH and Wnt compared with the early follicular

Abbreviations: AR, androgen receptor; ER, estrogen receptor; FOXO, forkhead box protein O; FSH, follicle-stimulating hormone; HH, hedgehog; IQR, interquartile range; LH, luteinizing hormone; PI3K, phosphoinositide-3-kinase; RT-qPCR, real-time quantitative reverse transcription-PCR; SOD2, superoxide dismutase 2; STP, signal transduction pathway; TGF-β, transforming growth factor-beta; Wnt, canonical wingless-type MMTV integration site.

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phase (both $p = 0.016$). Signal transduction pathway activity in fimbrial epithelium from postmenopausal patients was most similar to the early follicular and/or late luteal phase with regard to the AR, ER and PI3K pathways. Wnt pathway activity in postmenopausal patients was comparable to the late follicular and early luteal phase. We observed no differences in HH and TGF-β pathway activity between pre- and postmenopausal samples. The cyclic changes in signal transduction pathway activity suggest a stage-specific function which may affect the morphology and physiology of the human fallopian tube.

Conclusions: We demonstrated cyclic changes in activity of the AR, ER, PI3K, HH and Wnt pathways throughout the hormonal cycle.

KEYWORDS
computational models, fimbriae, hormonal cycle, human fallopian tube, signal transduction pathways

1 | INTRODUCTION

The fallopian tube has essential roles in human reproduction as it facilitates ovum and semen transport, fertilization and early embryonic development.[1] Due to its close proximity, the fallopian tube epithelium is regularly exposed to changing hormone levels produced by the ovary.[2] At the time of ovulation, when the oocyte is expelled from the dominant follicle, the fimbrial epithelium is embedded in pro-inflammatory follicular fluid.[2] There is evidence that exposure to follicular fluid induces tissue injury and altered gene expression in the fimbrial epithelium.[3] As a result, the fallopian tube epithelium must be able to restore tissue homeostasis to prevent irreversible damage.

Cascades of protein interactions, named signal transduction pathways (STPs), are known to regulate many cellular processes including cell proliferation, differentiation and survival.[4] During the hormonal cycle, physiological changes in the morphology and function of the fallopian tube epithelium are thought to be regulated by signal transduction pathway activity. Previous studies indicated a role for the androgen (AR) and estrogen receptor (ER)[5] and Hedgehog (HH)[6] pathways in normal epithelial homeostasis, whereas aberrant activity of the phosphoinositide-3-kinase (PI3K),[7] transforming growth factor-beta (TGF-β)[8] and canonical wingless-type MMTV integration site (Wnt)[9] pathways were associated with processes leading to female subfertility.

Interest in the fallopian tube epithelium is growing as the local environment of the fallopian tube represents the optimal conditions for the reproductive process. Establishing knowledge of normal STP activity in the fallopian tube epithelium is pivotal in the understanding of reproductive aspects, as well as aberrant STP activity in tubal diseases. However, the molecular processes that affect gene expression in the fallopian tube during the hormonal cycle remain poorly understood. To measure functional STP activity, Verhaegh et al.[10] developed a technique to quantitatively measure activity of the above-mentioned STPs based on mRNA levels of pathway-specific target genes. In this study we investigate the activity of key STPs in fimbrial epithelium of morphologically normal fallopian tubes from pre- and postmenopausal patients.

Key message

The molecular processes that affect gene expression in the fallopian tube remain poorly understood. This study demonstrates cyclic changes in the activity of key signal transduction pathways in fimbrial epithelium of human fallopian tubes during the hormonal cycle.

2 | MATERIAL AND METHODS

2.1 | Study population

We included healthy premenopausal patients ($n = 17$) who participated in the HYSTUB randomized controlled trial in which patients were randomized to undergo hysterectomy either with or without concurrent salpingectomy (NCT02281487).[11] Surgical indications included heavy menstrual bleeding ($n = 6$), uterine leiomyomas ($n = 5$), cervical dysplasia ($n = 3$) and abdominal pain ($n = 3$). In addition, we included healthy fallopian tubes from postmenopausal patients (aged >55 years, $n = 8$) obtained during surgical interventions for benign gynecologic conditions identified by the Dutch national pathology archive (PALGA) between 2009 and 2018. These patients had salpingectomy with or without hysterectomy for a benign ovarian mass ($n = 5$), postmenopausal blood loss without (pre)malignancy ($n = 2$) and uterine descensus ($n = 1$). We excluded patients with a history of gynecologic cancer prior to surgery (except for cervical dysplasia), known pathogenic BRCA 1/2 germline mutations and patients with a positive family history of any type of hereditary cancer.

Premenopausal patients did not use any hormones 3 weeks prior to surgery, as this was an exclusion criterion for participation in the HYSTUB trial. None of the patients with uterine leiomyomas were treated with gonadotropin-releasing hormone agonists. For postmenopausal patients, we screened medication history for the use of hormones prior to surgery in order to prevent exogenous hormonal influences. Demographic data including age at surgery, parity, body mass index (BMI) and data on a history of subfertility, ectopic pregnancy, tubal sterilization, endometriosis and adenomyosis were extracted from medical records.

2.2 | Determination of hormonal cycle phase

Histologic sections of the endometrium of premenopausal patients were reviewed by two independent gynecologic pathologists (M.H.F.M.L.-B. and S.L.B.) according to predefined characteristics to determine the hormonal cycle phase.[12] As part of the HYSTUB trial, premenopausal patients were subjected to blood sample collection to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol serum levels either 1 day before or on the day of surgery.[11] Hormone concentrations and endometrial characteristics were matched to determine hormonal cycle phase. For the premenopausal patients, we decided to characterize the hormonal cycle into four phases (either early/late follicular or early/late luteal) to determine subtle differences in STP activity.

2.3 | Laser capture microdissection

Histologic sections of the fallopian tubes were reviewed by a pathologist (M.H.F.M.L.-B. or S.L.B.) and morphologically normal fimbriae were annotated. Formalin-fixed paraffin-embedded sections of 5 μm were cut on PEN membrane slides (article number 415190-9041-000, Carl Zeiss B.V., Germany) with a microtome (RM2255, Leica Biosystems, Germany). Slides were manually hematoxylin stained. To avoid stromal contamination, fallopian tube epithelium was harvested using a laser microdissector (PALM MicroBeam 4, Carl Zeiss B.V., Germany).

2.4 | mRNA extraction and real-time PCR analysis

After laser capture microdissection, total mRNA was extracted according to the manufacturer's protocol (VERSANT® Tissue Preparation Reagents kit, Siemens, Germany). Real-time quantitative reverse transcription-PCR (RT-qPCR) was performed using the SuperScript™ III Platinum™ One-Step qRT-PCR kit (Invitrogen, ThermoFisher Scientific, USA). Commercially available OncoSignal 96-well PCR plates (Philips MPDx, The Netherlands) were processed with a CFX96 Real-Time PCR Detection System (Bio-Rad, USA). Internal quality control of reference genes confirmed sufficient input for pathway analysis.

2.5 | Signal transduction pathway assays

With the use of commercially available RT-qPCR-based OncoSignal pathway assays (Philips MPDx, The Netherlands), STP activity of the following pathways was measured: the AR, ER, PI3K (indirectly, as it is inversely proportional to Forkhead Box Protein O [FOXO] transcription factor activity; on the premise that that no cellular oxidative stress is present, as described before),[13] HH, TGF-β and Wnt pathway. The assays quantitatively measure activity of these signaling pathways using Bayesian network computational models which infer activity of the corresponding transcription factor complex from the expression of pathway-specific target genes. The approach has been described in detail before.[10] Originally, the models were developed and validated on multiple cell types using Affymetrix expression microarray data and included approximately 25–35 target genes per pathway.[13-15] The selected target genes corresponding to the transcription complex of the ER and Wnt pathways,[10] AR, HH and TGF-β pathways[14] and PI3K pathway[13] have been described in detail previously. To facilitate clinical application, the models were adapted based on a selection of the best performing target genes (around 12 target genes) to enable the use of RT-qPCR mRNA measurements from formalin-fixed paraffin-embedded material.[16-18] A major advantage of the assays is the reliable readout of direct target genes of the respective pathway-associated transcription factor because increased expression levels are direct evidence of pathway activation. Conventional methods such as immunohistochemistry or immunoblotting can identify signaling proteins or transcription factor proteins but do not provide reliable and quantitative information on the functional activity state of the proteins and therefore are not suitable to infer associated STP activity. The assays present functional pathway activity scores on a normalized 0–100 scale, where 0 theoretically corresponds to the lowest and 100 to the highest odds in favor of an active pathway; however, the actual activity range may be restricted to a certain part of this 0–100 scale, depending on the specific STP and the cell type in which it is measured, as explained before.[14] Given the cell type-specific STP range, direct comparison of the activity scores of the different STPs is not possible. However, once the STP range has been defined, the scores of every new sample can be interpreted against the defined range to classify normal or aberrant activity.

2.6 | Statistical analyses

Patient characteristics in pre- and postmenopausal groups were compared using independent *t* tests or Wilcoxon rank sum tests. Differences in STP activity between hormonal phase groups were tested with pairwise comparison using Wilcoxon rank sum exact tests as the analyses were considered exploratory. Correlations were assessed using Spearman's rank correlation coefficient. The *p* values <0.050 were considered statistically significant. Statistical analysis and data visualization was conducted using R (version 1.1.463).

2.7 | Ethical approval

The study was approved by the Medical research Ethics Committees United (MEC-U, study number W18.134, 29 August 2018). All patients gave their written informed consent and tissues were studied in accordance with the Code of Conduct of the Federation of Medical Scientific Societies in The Netherlands.

3 | RESULTS

3.1 | Study characteristics

Median age at surgery was 44 years (interquartile range [IQR] 41–47) and 62 years (IQR 59–67) for the pre- and postmenopausal groups, respectively ($p < 0.001$) (Table 1). We observed no statistically significant differences between the pre- and postmenopausal groups regarding BMI, parity, subfertility, ectopic pregnancy, tubal sterilization, endometriosis or adenomyosis. Evaluation of hormone serum levels and histologic sections of the endometrium resulted in four early follicular, three late follicular, five early luteal and five late luteal samples. Representative histologic images of each hormonal phase are presented in Figure S1. Hormone serum levels per phase of the hormonal cycle are presented in Figure 1 and show that the late follicular phase was characterized by high FSH and LH concentrations whereas the early luteal phase demonstrated lower estradiol concentrations compared with the late follicular phase, as described previously.[19] In the early follicular phase, one patient had a markedly higher estradiol serum level in relation to the other premenopausal patients. This patient was characterized by a relatively high BMI, which may have resulted in elevated estradiol production by adipose tissue. However, we observed lower estradiol levels in patients with higher BMI. Although information in the patients' medical records and medication history did not provide an explanation for the elevated estradiol level, we decided to include this patient in our cohort given our small sample size. However, we marked the sample of this patient in the following analysis. Additional clinical details and pathological findings are described in Table S1.

TABLE 1 Patient characteristics of the pre- and postmenopausal groups

Variables	Premenopausal ($n = 17$)	Postmenopausal ($n = 8$)	p value
Age at surgery (years)			<0.001
Median (IQR)	44 (41–47)	62 (59–67)	
BMI (kg/m^2)			0.457
Mean (SD)	26.4 (3.4)	27.5 (3.8)	
Parity (number)			0.109
Median (IQR)	2 (1–3)	2 (2–4)	
Subfertility			1.000
Yes (%)	2 (12%)	0 (0%)	
No (%)	15 (88%)	8 (100%)	
Ectopic pregnancy			1.000
No (%)	17 (100%)	8 (100%)	
Tubal sterilization			1.000
Yes (%)	4 (24%)	1 (12%)	
No (%)	13 (76%)	7 (88%)	
Endometriosis			0.527
Yes (%)	3 (18%)	0 (0%)	
No (%)	14 (82%)	8 (100%)	
Adenomyosis			0.411
Yes (%)	10 (59%)	3 (37%)	
No (%)	7 (41%)	5 (63%)	
Hormonal cycle phase			
Early follicular	4 (24%)	—	—
Late follicular	3 (18%)	—	—
Early luteal	5 (29%)	—	—
Late luteal	5 (29%)	—	—

Abbreviations: BMI, body mass index; IQR, interquartile range; SD, standard deviation.

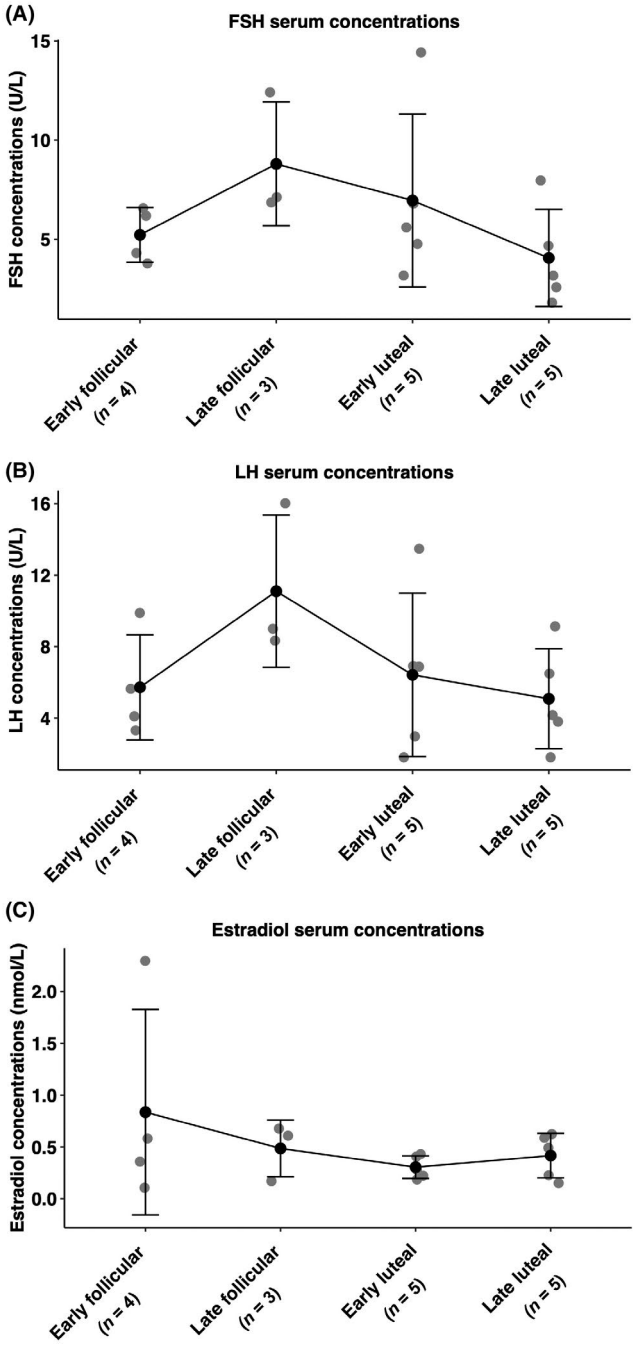

FIGURE 1 Line graphs displaying hormone serum concentrations in premenopausal patients during the menstrual cycle. (A) Follicle-stimulating hormone (FSH) serum concentrations (U/L). (B) Luteinizing hormone (LH) serum concentrations (U/L). (C) Estradiol serum concentrations (nmol/L). Black dots with error bars represent mean concentrations with standard deviations. Gray dots indicate individual patients. Note that in the early follicular phase there is one patient with markedly higher estradiol concentrations, which strongly influenced the mean concentration during this phase. Repeated analysis after exclusion of this patient demonstrated higher estradiol concentrations during the late follicular and late luteal phase when compared with the early follicular and early luteal phase

3.2 | Signal transduction pathway activity in the human fallopian tube during the hormonal cycle

Our results demonstrate gradual differences in STP activity of the AR, ER, PI3K, HH and Wnt pathways in fallopian tube epithelium during the hormonal cycle (Figure 2).

Throughout the follicular phase, we observed minimal changes in STP activity without statistically significant differences, possibly due to the small sample number. The early follicular phase was distinguished by higher HH, TGF-β and Wnt pathway activity followed by a slight decrease towards the late follicular phase. In addition, a slight increase in AR and PI3K pathway activity was observed towards the late follicular phase.

The early luteal phase was characterized by peak activities in AR and ER pathway activity and low PI3K pathway activity. AR pathway activity was significantly higher during the early luteal phase than during the early follicular phase ($p = 0.016$), whereas PI3K pathway activity was significantly lower during the early luteal phase than during the late follicular phase ($p = 0.036$). In one of the early luteal samples, we observed high FOXO activity in conjunction with high superoxide dismutase 2 (SOD2) expression. The *SOD2* gene codes for a protein that protects against oxidative damage.[13] This may indicate cellular oxidative stress as an alternative cause of FOXO activity. Therefore, in this sample, PI3K pathway activity should be interpreted with caution. Repeated analysis after exclusion of this patient still demonstrated the lowest PI3K pathway activity in the early luteal phase but with borderline significance compared with the late follicular phase ($p = 0.057$). ER pathway activity during the early luteal phase was not significantly different from the follicular phase, which was likely to be influenced by the outlier with low ER pathway activity in the early luteal phase. Consequently, exclusion of this sample showed a significant difference between the early follicular and early luteal phase ($p = 0.029$).

Towards the late luteal phase, both the AR and ER pathways demonstrated a distinct decrease ($p = 0.016$ and $p = 0.032$, respectively). The decrease in TGF-β pathway activity between the early luteal phase and late luteal phase reached borderline significance ($p = 0.056$). Activity of the Wnt pathway was significantly lower during the late luteal phase than during the early luteal phase ($p = 0.016$), whereas both the HH and Wnt pathways demonstrated significantly lower activity during the late luteal phase than during the early follicular phase (both $p = 0.016$).

The STP activity in fallopian tube epithelium from postmenopausal patients showed the greatest similarities with the early follicular and/or late luteal phase regarding the AR, ER and PI3K pathways. We observed significant differences in AR and ER pathway activity when comparing the postmenopausal samples with the early luteal phase (respectively $p = 0.002$ and $p = 0.040$), whereas there was a difference in PI3K activity in postmenopausal samples from the late follicular phase ($p = 0.048$) and early luteal phase ($p = 0.019$). Wnt pathway activity in postmenopausal samples was similar to the

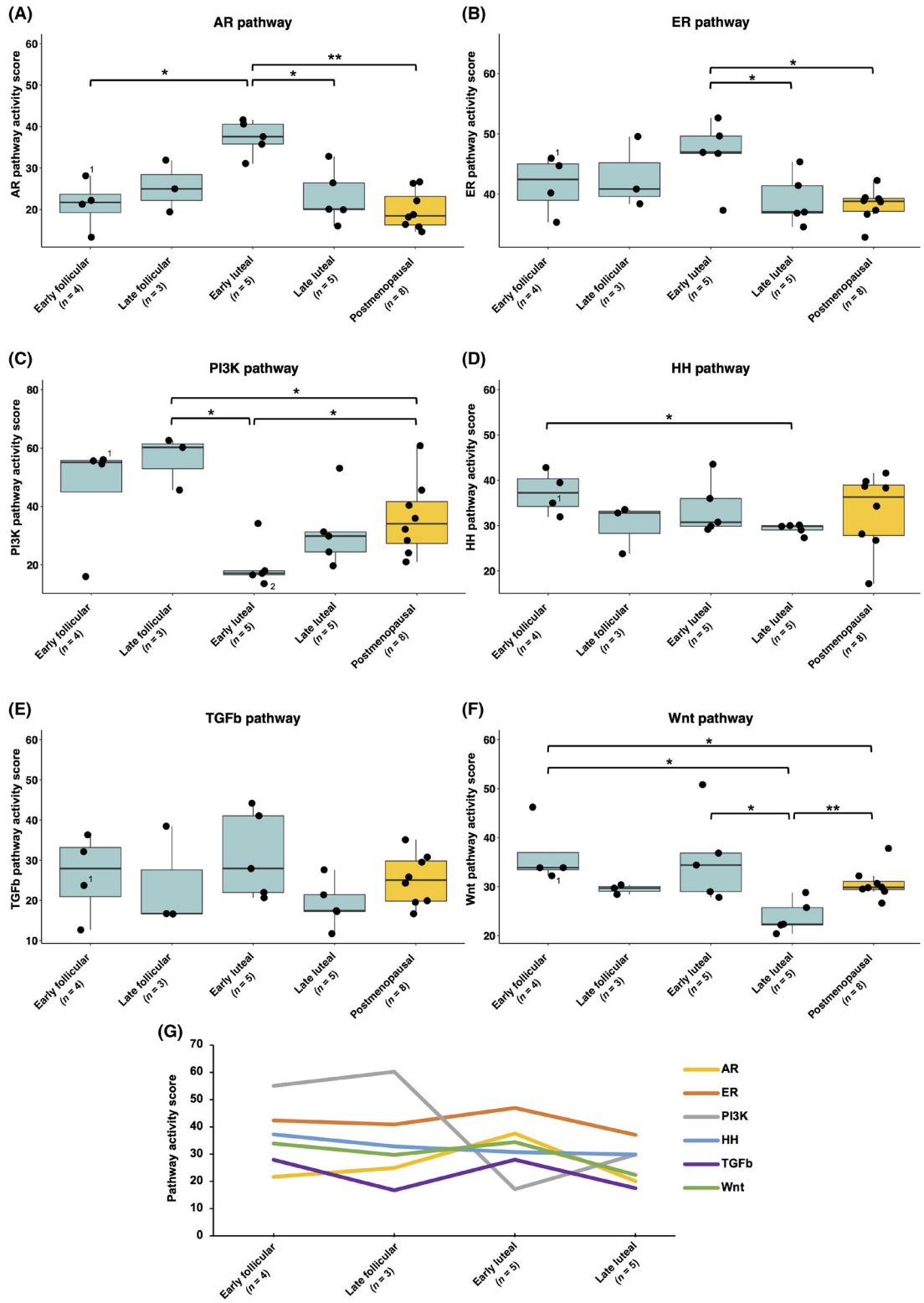

FIGURE 2 Signal transduction pathway (STP) activity in fimbrial epithelium of fallopian tubes from pre- and postmenopausal patients. (A) Androgen receptor (AR). (B) Estrogen receptor (ER). (C) Phosphoinositide-3-kinase (PI3K). (D) Hedgehog (HH). (E) Transforming growth factor beta (TGFb). (F) Canonical wingless-type MMTV integration site (Wnt). (G) Simplified overview of median STP activity scores measured during the menstrual cycle. Direct comparison of the activity scores of the different STPs was not possible, as every STP has his own cell type-specific range of STP activity. *$p < 0.050$; **$p < 0.010$; [1]Patient with a relatively high estradiol serum concentration. Repeated analysis after exclusion of this patient demonstrated minimal changes in significance levels without influencing our conclusions. [2]Sample with evidence for oxidative stress

late follicular and early luteal phase, as the activity clearly differed from that during the early follicular ($p = 0.028$) and late luteal phase ($p = 0.003$). For the HH and TGF-β pathways, we observed no differences in STP activity between the pre- and postmenopausal samples.

Subsequently, we examined whether estradiol serum levels were associated to functional activity of the hormonal pathways. For both ER and AR pathway activity, we observed no statistically significant correlation with estradiol levels (Spearman R = 0.150, $p = 0.550$ and Spearman R = 0.037, $p = 0.890$, respectively). However, a positive correlation was found between and ER and AR pathway activity (Spearman R = 0.620, $p = 0.001$).

4 | DISCUSSION

This is the first study to investigate functional STP activity in the human fallopian tube epithelium. Our results demonstrate that STP activity in fallopian tube epithelium changes during the hormonal cycle. Specifically, the early luteal phase showed high AR and ER pathway activity compared with the late luteal phase, and low PI3K activity compared with the late follicular phase. On contrast, the late luteal phase showed low activity of the HH and Wnt pathways compared with the early follicular phase (Figure 2). The STP activity in fallopian tube epithelium from postmenopausal patients was most similar to the early follicular and/or late luteal phase with regard to the AR, ER and PI3K pathways. Wnt pathway activity in postmenopausal patients was comparable to that in the late follicular and early luteal phase.

Previously, presence of the androgen and estrogen hormone receptors has been demonstrated by immunohistochemistry in fallopian tube epithelium of both pre- and postmenopausal patients, suggesting hormonal responsiveness.[5] We observed a positive correlation and comparable pattern of gradual differences in AR and ER pathway activity in fallopian tube epithelium during the course of the hormonal cycle (Figure 2A,B). However, the pattern of AR and ER pathway activity did not resemble the measured fluctuations in estradiol serum levels (Figure 1C). In line with previous evidence indicating decreased estradiol serum levels after ovulation, we observed lower estradiol levels during the early luteal phase compared with the late follicular phase.[19] Interestingly, in fallopian tube epithelium, the highest ER and AR pathway activity was observed during the early luteal phase. With no significant correlation between estradiol serum levels and ER or AR pathway activity, our findings show that pathway activity in fallopian tube epithelium is not sufficiently reflected by serum ligand availability. In addition, it is more likely that the fallopian tube epithelium is influenced by the local paracrine function of sex steroid hormones. After ovulation, the fimbriae are exposed to follicular fluid released from the dominant follicle. Follicular fluid is mainly composed of steroid hormones, growth factors, cytokines and reactive oxygen species.[20] In relation to serum levels, extremely high levels of estradiol have been measured in follicular fluid.[21] Therefore, after ovulation the fimbriae are embedded in an environment enriched with steroid hormones, which may contribute to the prolonged high AR and ER pathway activity after ovulation.

Surprisingly, we found preserved AR and ER pathway activity in postmenopausal samples. As the ovaries cease to produce hormones after menopause, this suggests a role for hormonal signaling irrespective of circulating serum levels.[22] Although residual hormones in postmenopausal patients are produced by extragonadal conversion in peripheral tissues, for instance in vascular endothelium, brain, bone and adipose tissue, these hormones predominantly act on local tissues.[23] Alternatively, our data may suggest that the fallopian tube epithelial cells have the ability to synthesize hormones and act as an intracrine factor to maintain intracellular hormone metabolism. Thereby the epithelial cells may actively modify their hormonal signaling without being dependent on endocrine or paracrine concentrations. On this basis, it is reasonable to expect that fallopian tube epithelium would express aromatase activity to facilitate local estrogen biosynthesis from circulating precursors, as is the case in peripheral tissues.[24] Although aromatase activity has been reported in oviductal epithelium of mammals,[25] a study with human fallopian tubes of premenopausal patients failed to identify aromatase expression.[26] However, there is supportive evidence for intracrine biosynthesis via alternative enzymes, for example conversion of the precursor estrone sulfate using steroid sulfatase.[27] So far, studies investigating intracrinology of the fallopian tube have been underrepresented in previous literature, suggesting focus of further studies on this area of investigation.

Another finding of our study was high PI3K pathway activity during the follicular phase compared with the luteal phase (Figure 2C). Considering that the PI3K pathway is central to the control of cell proliferation, metabolism and survival, this might suggest a role in regulation of morphologic changes of the epithelium. In the endometrium, the downstream effectors of PI3K, such as AKT, have been shown to influence cell motility.[28] Moreover, endometrial decidualization, which occurs during the luteal phase under influence of progesterone, is characterized by decreased activity of the PI3K/AKT pathway.[29] Progesterone is able to decrease PI3K/AKT signaling in endometrial cells, as progesterone receptor signaling induced FOXO transcription factor activity and thus caused decreased PI3K activity.[30] These findings are in line with our results, as we observed a distinct decrease in PI3K pathway activity in fallopian tube epithelium in the luteal phase.

As mentioned, tight regulation of (in)activation of STP activity may be important to support morphologic changes of the epithelium. Such transformations include increased mitotic activity and height of ciliated and secretory cells during the follicular phase stimulated by estrogens to facilitate successful ovum and semen transport, and deciliation and atrophy during the luteal phase which is associated with high levels of progesterone.[31] After menopause, the percentage of ciliated cells significantly decreases with loss of secretory activity.[32] There is supportive evidence that primary ciliary expression helps coordinate several signaling pathways, including TGF-β, HH and Wnt signaling, as mediators by receiving extracellular signals.[33-35] Besides that, the formation of cilia was also found to be regulated by signaling activity.[33] A study investigating primary cilia on secretory cells of the

fallopian tube demonstrated that primary cilia were responsive to HH signaling, suggesting this pathway is involved in ciliary function.[6] Moreover, murine experiments confirmed a role for Wnt signaling in oviductal epithelial homeostasis, as the addition of a Wnt inhibitor to an oviduct culture model resulted in a decreased embryo transport distance.[36] Others confirmed that conditional overactivation of β-catenin, an important signaling member of the Wnt pathway, resulted in expansion of secretory cells, whereas ablation of β-catenin reduced the proportion of secretory cells.[37] Our findings showed an identical pattern in HH and Wnt pathway activity, wherein the early follicular phase showed higher activity compared with the late luteal phase (Figure 2D,F). Unfortunately, our results reflect pathway activity in both ciliated and secretory cells, making it difficult to uncover a cell type-specific function during the hormonal cycle. Nevertheless, detailed characterization of these pathways might help explain diseases associated with ciliary dysfunction and, consequently, female subfertility.

The major strength of this study is the quantitative measurement of functional activity of several key STPs. Conventional methods, such as immunohistochemical staining, are limited by the identification of individual proteins, and the presence of a protein does not automatically imply that the complete STP is activated. With the use of the pathway assays based on mRNA levels of pathway-specific target genes we were able to measure functional STP activity. A limitation of our study is its retrospective nature, as we were dependent on the available data on hormone serum concentrations, which lacked information on progesterone levels in both groups and estradiol levels in the postmenopausal group to validate hormonal cycle phase. Moreover, the study was limited by the small sample size and the unequal distribution of inclusions in the different groups of the hormonal cycle, as this study was designed as an exploratory study. It is possible that subtle changes in STP activity which lacked statistical significance due to small sample number, for example during the follicular phase, may still be of biological importance. However, we observed notable patterns in STP activity, which justifies future studies with expanded sample size to define the fallopian tube-specific range of STP activity during the hormonal cycle.

Future research should compare STP activity in the different anatomic regions, as we only investigated the fimbrial epithelium, and study the cell type-specific STP activity of both ciliated and secretory cells. While the pathway assays have specifically been developed to measure functional STP activity, it would be interesting to supplement the results with information on the respective function of the activated pathway in terms of changes in tubal morphology. Cellular changes ultimately depend on the final product of an activated STP, namely, the synthesized proteins. Unfortunately, mRNA expression levels are unreliable indicators of corresponding protein expression due to an unequal distribution of production and turnover. Therefore, the use of protein expression analysis (ie immunohistochemistry or immunoblotting) might be considered. To further assess the functional relevance of an activated pathway, we suggest the use of ex vivo oviduct cultures or organoids, which allow experimental conditions for investigating the effect of pathway inhibitors and activators on protein expression and determining morphologic changes of the tubal epithelium. Such experiments not only have the potential to improve our understanding of the molecular processes occurring in the fallopian tube but are also crucial to identify possible therapeutic targets in the case of tubal diseases, for example to improve tubal patency in case of obstruction or to treat tubal carcinogenesis.

5 | CONCLUSION

We observed cyclic changes in STP activity in human fallopian tube epithelium during the hormonal cycle, the early luteal phase characterized by high AR and ER pathway activity and low PI3K pathway activity and the late luteal phase by low HH and Wnt pathway activity. AR, ER and PI3K pathway activity in fallopian tube epithelium from postmenopausal patients was most comparable to the activity measured during the early follicular and/or late luteal phase. Wnt pathway activity in postmenopausal patients was comparable to the late follicular and early luteal phase. The cyclic changes in STP activity suggest a stage-specific function which may affect the morphology and physiology of the human fallopian tube.

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CONFLICT OF INTEREST

Phyllis van der Ploeg is an employee of the Catharina Hospital, where her research work is funded by the Catharina Research fund and Molecular Pathway. Dx, Philips. Anja van de Stolpe is an employee of Molecular Pathway Dx, Philips. The other authors declare no conflict of interest.

AUTHOR CONTRIBUTION

P.v.d.P.: conception and design, acquisition of data, analysis and writing. A.U. and K.M.M.B.: acquisition of data and analysis. M.H.F.M.L.-B., S.L.B., M.M.E.v.R., M.C.V., P.J.v.D. and S.L.: acquisition of data. A.v.d.S.: conception and design and analysis. R.L.M.B. and J.M.J.P.: conception and design, acquisition of data and supervision. All authors contributed to data interpretation, revised the manuscript critically for intellectual content and approved the submitted version.

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SUPPORTING INFORMATION

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