# Fallopian tube epithelial cells express androgen receptor and have a distinct hormonal responsiveness when compared with endometrial epithelium

**Running title:** Hormonal responsiveness of the fallopian tube

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**Abstract**

**Study question:** How does steroid receptor expression, proliferative activity and hormone responsiveness of the fallopian tube (FT) epithelium compare to that of the endometrial epithelium?

**Summary answer:** Proliferative indices, hormone receptor expression-scores and *in-vitro* response to oestrogen and androgens of the human FT demonstrate a distinct pattern from the matched endometrium.

**What is known already:** The FT epithelium exists as a continuum of the endometrium, and both express steroid hormone receptors. The ovarian steroid hormones regulate cyclical proliferation and regeneration of the endometrium, but their effects on steroid hormone receptor expression and proliferation in the FT have not yet been fully elucidated.

**Study design, size, duration:** We included normal women with proven fertility undergoing hysterectomy and bilateral salpingo-oophorectomy for benign gynecological conditions at Liverpool Women’s NHS Foundation Trust. They had no known endometrial or tubal pathology and were not on hormonal treatments for at least 3 months preceding sample collection in this prospective observational study (conducted between 2010-2018). A full-thickness sample of the endometrium and a sample from the FT were collected from each woman.

**Participants/materials, setting, methods:** The differential protein and mRNA levels of steroid hormone receptors, oestrogen receptors alpha (ERα) and beta (ERβ), androgen receptor (AR) and progesterone receptor (PR), and the proliferative marker (Ki67) of the endometrium and the FT tissue samples from 47 healthy women undergoing surgery (37 premenopausal and 10 postmenopausal) were investigated using immunohistochemistry and qPCR. The comparative responsiveness to oestrogen and androgen of the endometrium and the fimbrial end of the FT was analysed using an *in-vitro* short-term explant culture model. The end points assessed in the explants were the changes in mRNA and protein levels for AR, PR, and the epithelial proliferative index after 24h treatment with oestradiol (E2) or dihydrotestosterone (DHT).

**Main results and the role of chance:** The pre-menopausal endometrial functional glands (FG) displayed the well-known cyclic variation in cellular proliferation and steroid receptor scores. Compared with the endometrial FG, the matched FT epithelium (both fimbrial or isthmic ends) displayed a significantly lower proportion of cells expressing Ki67 (2.8% ± 2.2%, n=18 versus 30.0% ± 26.3%,n=16, *P*=0.0018, respectively) accompanied with a significantly higher AR immunoscores (6.7 ± 2.7 n=16 versus 0.3 ±1.0 n=10, *P*=0.0136). The proportion of cells expressing Ki67 and the AR immunoscores of the FT epithelium correlated positively with endometrial luminal epithelium (r=0.62, *P*=0.005, and r=0.68, *P*=0.003, respectively). *In-vitro* experiments suggested the tubal explants to be apparently less responsive to E2 yet more sensitive to DHT compared with the matched endometrium explants.

**Limitations,** **reasons for caution:** The short-term *in vitro* nature of the tissue explant cultures used in the study may not be representative of how different anatomical regions of the endometrium and FT behave *in vivo*. Our study included a high proportion of older premenopausal women with a regular menstrual cycle, which may therefore affect extrapolation of findings to a younger group.

**Wider implications of the findings:** Advancing our understanding of tubal and endometrial epithelial cell function has important implications for the diagnosis and treatment of diseases such as infertility, ectopic pregnancy, endometriosis and cancer.

**Study funding/competing interest(s):** The work included in this manuscript was funded by Wellbeing of Women project grants RG1073 and RG2137 (D.K.H.) and Wellbeing of Women Entry-Level Scholarship ELS706 (A.M). AM was also supported by a NIHR ACF fellowship grant. Further support received from Liverpool Women’s Hospital NHS Trust (S.M.), University of Liverpool (E.B., A.W.). All authors declare there are no conflicts of interest.

**Trial registration number:** n/a

Keywords: Endometrium, fallopian tube, steroid receptors, proliferation, hormone responsiveness, androgen receptor

# Introduction

The epithelium of the human fallopian tube (FT) exists as a continuum of the endometrium, the mucosa lining the uterine cavity and both tissues share the same embryological origin ([Indumathi et al. 2013](#_ENREF_15); [Hill CJ 2020](#_ENREF_12)). The endometrium is the major target organ of ovarian steroid hormones, and under their influence it undergoes a well-described cycle of proliferation, differentiation, shedding and regeneration throughout a woman’s reproductive life ([Hapangama, Kamal, and Bulmer 2015](#_ENREF_10)). This hormonal regulation is initiated via the cognate hormone receptors; oestrogen receptors α (ERα) and β (ERβ), progesterone receptor (PR) and androgen receptor (AR), which are expressed in endometrial cells ([Hapangama, Kamal, and Bulmer 2015](#_ENREF_10)). Endometrial glands proliferate in response to rising follicular oestrogen levels, mainly oestradiol (E2) ([Kamal, Tempest, et al. 2016](#_ENREF_17)). There is *in vitro* and *in vivo* evidence that E2 induces endometrial epithelial proliferation via ERα, whilst also increasing the expression of other steroid receptors ([Hapangama, Kamal, and Bulmer 2015](#_ENREF_10); [Parkes et al. 2018](#_ENREF_28); [Krikun et al. 2005](#_ENREF_19)). The dominant hormone of the luteal phase, progesterone (P), acts via the PR to counteract the effects of oestrogen and inhibits endometrial glandular proliferation ([Maybin and Critchley 2011](#_ENREF_24); [Hapangama, Kamal, and Bulmer 2015](#_ENREF_10)). *PR* is a known oestrogen-responsive gene ([Savouret et al. 1991](#_ENREF_33)), regulated by ERα activity ([Brandenberger et al. 1999](#_ENREF_2); [Couse and Korach 1999](#_ENREF_5)). The third ovarian hormone, androgen, acts via the AR. *AR* gene is known to be upregulated by both E2 via ERα ([Hapangama, Kamal, and Bulmer 2015](#_ENREF_10); [Brosens et al. 2004](#_ENREF_3)) and by androgens via AR ([Lovely et al. 2000](#_ENREF_22); [Fujimoto et al. 1994](#_ENREF_7)).

The cycle-specific expression of the steroid receptors in the endometrium and the effects of the ovarian steroid hormones on endometrial epithelium are well-described ([Hapangama, Kamal, and Bulmer 2015](#_ENREF_10); [Kamal, Tempest, et al. 2016](#_ENREF_17); [Kamal, Bulmer, et al. 2016](#_ENREF_16)). Previous authors have demonstrated that FT epithelium expresses the hormone receptors ER, PR, and AR ([Horne et al. 2009](#_ENREF_13); [Shao et al. 2011](#_ENREF_34); [Wilson and McPhaul 1996](#_ENREF_39)) suggesting that the FT may be responsive to ovarian steroid hormones. Further, previous studies have suggested ER and PR expression to be similar in different anatomical regions of the FT (ampullary, isthmic, fimbrial) ([Christow, Sun, and Gemzell-Danielsson 2002](#_ENREF_4); [Pino et al. 1982](#_ENREF_30); [Punnonen and Lukola 1981](#_ENREF_31); [Sun et al. 2003](#_ENREF_36)). However, the temporal and spatial expression of the receptors and the effect of steroid hormones on the expression of these receptor genes in FT epithelium at different stages of the menstrual cycle is yet to be fully elucidated.

The main objective of our work was therefore to examine the distinct steroid hormone receptor expression pattern and hormone responsiveness of the human FT epithelium, compared with the known hormone regulation of matched endometrial tissue. To this end, we initially compared the differences in proliferative indices and the steroid receptor immuno-expression pattern in the FT epithelium (fimbrial/isthmic portions) and the matched endometrial epithelial sub-compartments, of premenopausal women across the menstrual cycle, and of postmenopausal (PM) women. The differences in steroid receptor gene expression were analysed by qPCR. We then examined the differential *in vitro* hormone responsiveness of the two mucosal layers by using a short-term explant culture model of the fimbrial end of the tubal mucosa and endometrium exposed to E2 and the naturally occurring potent androgen, dihydrotestosterone (DHT).

# Materials and Methods

**Patient population**

Patient demographics are detailed in Table I. A total of 47 normal matched FT and full-thickness endometrial biopsies were collected from women undergoing hysterectomy and bilateral salpingo-oophorectomy in Liverpool Women’s NHS Foundation Trust between 2010 and 2018. Indications for hysterectomy included fibroids, pelvic organ prolapse, and heavy menstrual bleeding. Patients with known endometrial or tubal pathologies as well as those with a history of endometriosis, adenomyosis or ectopic pregnancies were excluded. Premenopausal women included had regular periods and the samples were assigned to a menstrual cycle phase using both patient reported last menstrual period date and morphological criteria, dated according to recent modifications of Noyes criteria, by two experienced pathologists ([Murray et al. 2004](#_ENREF_26)) as previously described (Kamal, Bulmer et al. 2016). All women included in the study were not on hormonal treatments for at least 3 months preceding sample collection. A wedge shaped section of full-thickness endometrium from the lumen to the myometrium, including functional and basal layers of the endometrium, and matched FT samples (fimbrial end and isthmic end) were obtained, from each of the hysterectomy specimens, from the same woman. Each sample was split into two (or 3 in 7 women), one was fixed in 10% neutral buffered formalin (NBF) for 24 hours and paraffin-embedded for immunohistochemical staining, and the other (exclusively the fimbrial end, rich in epithelial cells) was placed into RNA*later* (Life Technologies, Inchinnin, UK) for RNA extraction and qPCR. For explant cultures, the full-thickness endometrium and matched fimbrial end of the FT from 7 women in mid/late proliferative phase of the cycle were also collected and stored in phenol-free Dulbecco’s Modified Eagle Medium (DMEM/F12) (Life Technologies, Paisley, UK). Patient’s clinical and demographic details were collected at the time of recruitment.

**Ethical approval for the collection and use of human tissue**

The collection of human tissue was approved by Liverpool Adult Research Ethics Committee (LREC: 09/H1005/55 and 11/H1005/4).

**Immunohistochemistry (IHC)**

Heat induced antigen retrieval in citrate buffer at pH 6 was performed on 3 µm formalin-fixed paraffin embedded tissue sections as previously described ([Hapangama et al. 2010](#_ENREF_11); [Hapangama et al. 2019](#_ENREF_9)) and tissue sections were subsequently immunostained with anti-human steroid receptor antibodies and Ki67. Antibody sources, concentrations and incubation details are given in Supplementary Table I. Antigen detection was performed using the ImmPRESS polymer-based system and visualization with the ImmPACT DAB (Vector Laboratories, Peterborough, UK). Matching isotype (0.5 µg/mL) replaced the primary antibody as a negative control, with internal positive controls performed in each run.

**Analysis of IHC staining**

Immunostaining for ERα, ERβ, AR, PR and Ki67 was analysed with reference to the three different sub-anatomical areas of the full-thickness endometrial samples: the luminal epithelium (LE), the functionalis glands (FG) and stroma, and basalis glands (BG) and stroma. In the FT, immunostaining was analysed independently in epithelial cells and stroma. Typically in a secretory phase sample, glands in the upper 2/3 of the endometrium below the LE, surrounded by sparse stroma was identified as the *functionalis* and glands in the lower 1/3 of the secretory phase endometrium adjacent to the endo-myometrial junction, surrounded by densely packed stroma was recognised as the *basalis*. The location of this basalis-functionalis boundary displayed the well-known variation according to the menstrual cycle phase. Percentage of Ki67 positive epithelial cells was calculated to ascertain the proliferative index and immunostaining for steroid receptors (ERα, ERβ, PR and AR) was assessed semi-quantitively using a four-tiered Liverpool endometrial steroid quick score (LESQS), as previously described ([Kamal, Bulmer, et al. 2016](#_ENREF_16)), which encompasses both staining intensity and abundance. All slides were coded, and scored blind by two independent observers, after which the code was broken and scores compared. Discrepancies between the two observers were resolved by re-evaluating the samples together and agreeing on a final score.

**Quantitative Real-Time Polymerase Chain Reaction (qPCR)**

Total RNA from endometrial and FT samples was extracted using TRIzol® Plus RNA Purification System (Life Technologies, Paisley, UK) according to the manufacturer’s instructions. The quantity of total RNA was determined by NanoDrop ND-1000 (ThermoFisher Scientific, UK). Total RNA was reverse transcribed using AMV First Strand cDNA synthesis kit (New England Bio Labs, Hertfordshire, UK). cDNA was amplified by qPCR using iTaq SYBR Green supermix and the CFX Connect Real-Time System (Bio-Rad, Hertfordshire, UK). Relative transcript expression was calculated by the ΔΔCT method, normalized to the reference gene GAPDH using the Bio-Rad CFX Manager Software 3.1. Primers and reaction conditions are listed in Supplementary Table II.

**Explant culture and hormone treatment**

Endometrial biopsies and matched tubes (n=7) were incubated for six hours in phenol red-free DMEM/F12 (Life Technologies, Paisley, UK), to remove blood and to eliminate the *in vivo* hormonal influence. The tissue was cut into 2-3 mm3 pieces and placed into 24-well plates with 2 mL of medium DMEM/F12, 5% (0.1ml) charcoal-stripped FBS (fetal bovine serum, Sigma-Aldrich, Dorset, UK), 100 µg/ml Primocin (Source Bioscience, Nottingham, UK). The samples were then incubated with either the hormone treatment, (10−8 M E2 (stock 1mg/ml in ethanol, Sigma-Aldrich, Dorset, UK) or 10−6 M DHT (stock 1mg/ml in methanol, Sigma-Aldrich, Dorset, UK)), or vehicle alone, for the controls, for 24 hours at 370C 5% CO2. Tissue was then collected into (i) RNA*later* (Sigma-Aldrich, Dorset, UK) and stored at 4 ºC for at least 24hours prior to removal of the RNA*later* and storage at −70 °C for qPCR and (ii) fixed in 10% NBF for 24 hours and paraffin-embedded for immunohistochemical staining.

**Statistical analysis**

All statistical analyses were performed using GraphPad Prism v 5.0, employing non-parametric tests (Kruskall-Wallis and/or Mann-Whitney U-test or Wilcoxon signed rank-test). Descriptive values are presented as mean ± SD unless otherwise stated. The correlations between immunoexpression scores and/or Ki67 were examined with Spearman’s rank tests. Results were considered statistically significant when *P*<0.05.

# Results

**The human FT demonstrates constitutively low levels of epithelial cell proliferation, significantly different to the matched endometrial functionalis glandular epithelium**

The menstrual cycle phase-associated differences in cellular proliferative activity in the endometrial and tubal epithelium were assessed by comparing their proliferative index (PI-Ki67, percentage of Ki67 immune-positive cells). There was no significant difference between the PI-Ki67 and all 4 steroid receptor immunoscores between the isthmic and fimbrial portion of the tubes (*P*>0.05). Matched full-thickness pre-menopausal endometrial samples containing all three endometrial epithelial layers and FT samples collected from the same woman were analysed. As expected, the pre-menopausal endometrial functional glands (FG) displayed the well-known cyclic variation in cellular proliferation, with highest PI-Ki67 observed in the oestrogen dominant proliferative phase (PP) (42.1% ± 18.2%, n=7). In comparison with all pre-menopausal endometrial FG samples, the matched FT epithelium displayed significantly lower PI-Ki67 (2.8% ± 2.2%, n=18 versus 30.0% ± 26.3%,n=16, *P*=0.0018, respectively) (Figures 1 and 2). In the endometrium, the cyclical phase variation in epithelial proliferation was also less prominent and significantly lower in the basalis (BG) and luminal (LE) region (for example in PP, PI-Ki67 in FG 22.2% ± 12.5%, n=9, in BG 15.6% ± 13.8%, n=7, in LE 42.1% ± 18.2%, n=7, *P*=0.0151). In hypo-oestrogenic PM samples, glandular epithelium also had significantly lower PI-Ki67 scores than the premenopausal FG (6.3% ± 11.9%, n=10, versus 29.7% ± 26.3%, n=16, *P*=0.024). Interestingly, the PI-Ki67 of the tubal epithelium correlated positively with endometrial LE (r=0.62, *P*=0.0005), but only weak correlation was identified with the FG other two endometrial epithelial regions (r=0.44, *P*=0.036) see Supplementary Table III.

**The tubal epithelial steroid receptor immuno-staining pattern is also distinct from the functional glands of the endometrium**

The steroid receptor expression levels in the endometrial samples in this study were consistent with the previously described cyclical variation in premenopausal functionalis and basalis glands, and steady immunoscores were observed in postmenopausal glands ([Kamal, Bulmer, et al. 2016](#_ENREF_16)) (Figures 2, 3, and Supplementary Figure 1). In contrast, the premenopausal FT epithelium lacked cyclical variation, and demonstrated a static immuno-reactivity for all four steroid receptors, regardless of the cycle phase. (Figures 2, 3, and Supplementary Figure 1).

The premenopausal endometrial epithelium demonstrated significantly low epithelial nuclear AR staining across all regions, when compared with matched tubal mucosa. AR immuno-reactivity was particularly weak in the FG and LE (0.3 ± 1.0 n=10, and 0.5 ± 0.7, n=5) of premenopausal endometrium, yet the PM endometrial epithelium demonstrated high AR immuno-scores in both the glands (4 ± 2.6, n=10) and in the luminal epithelium (7.9 ± 2.0, n=7) (Figures 2, and 3). The FT epithelium continually displayed high epithelial nuclear AR immunoscores in both premenopausal (6.7 ± 2.7, n=16), and in the PM samples (9.9 ± 2.7, n=8) (Figures 2 and 3). The premenopausal tubal epithelial AR immunoscores were significantly higher when compared with the matched endometrial FG (6.7 ± 2.7 n=16 versus 0.3 ±1.0 n=10, *P*=0.013) and this was persistently significant when PP and SP samples were considered separately (PP, FT 5.1 ± 3.4, n=7 versus FG 0 ± 0, n=4, *P*=0.031 and SP, FT 7.9 ± 1.3, n=9 versus FG 0.5 ±1.2, n=6, *P*=0.001, respectively). Although the endometrial LE immunoscores for AR were much lower than in the FT epithelium, they correlated positively with tubal epithelia AR scores (r=0.675, *P*=0.003), (Supplementary Table III). Furthermore, FT and LE, ERβ immunoscores were also positively correlated (r=0.524, *P*=0.018). (Supplementary Table III) Conversely, BG and FG epithelial ERα immunoscores negatively correlated with the ERα immunoscores of matched tubal epithelium (r=-0.561, *P*=0.002 and r=-0.56 and *P*=0.013, respectively), Supplementary Tables III-VII).

The endometrium, as expected, displayed cyclical *PR* mRNA expression, with a trend of higher expression in the PP compared to SP (1.302 ± 1.422 n=7, versus 0.445 ± 0.662 n=6, respectively), whilst the *AR* mRNA expression remained static. In contrast, the FT did not display cyclical variation in either *AR* or *PR* mRNA expression (Supplementary Figure 2A). Interestingly, *AR* and *PR* mRNA levels correlated positively in FT samples, which have a limited amount of stroma, thus epithelial contribution is high (r=0.647, *P*=0.0027). The mRNA levels for AR and PR also demonstrated a positive correlation in the endometrial samples, which is likely to have a greater contribution from the stromal compartment (r=0.739, *P*=0.0003) (Supplementary Figure 3).

Overall, stromal AR immuno-reactivity was significantly stronger in the endometrium than the matched FT (6.1 ± 3.71, n=30, versus 2.4 ± 3.2, n=10, *P*=0.013). In the premenopausal endometrium, AR immunoscores were higher in the stroma than the epithelium (6.38 ± 3.66, n=26 versus 0.79 ± 1.63, n=56, *P*=<0.0001). Conversely, AR immunoscores in the premenopausal FT were significantly stronger in the epithelium than the stroma (6.69 ± 2.77, n=16 versus 2.86 ± 3.72, n=7, *P*=0.024 (Supplementary Figure 4).

**Positive correlation between specific steroid receptor immunoscores and respective proliferative indices suggest a continuum between endometrial LE and FT epithelium**

Endometrial PR immuno-scores positively correlated with PI-Ki67 in the FG (r=0.83, *P*<0.0001) and LE (r=0.48, p=0.03) (Supplementary Table IV-VI). Interestingly, a positive correlation was observed between the endometrial LE and tubal epithelium PI-Ki67 (r=0.606, *P*=0.0005) AR (r=0.675, p=0.003) and ERβ immunoscores (r=0.524, *P*=0.018) (Supplementary Table III).

**The FT and the full-thickness endometrium respond differently to E2 and DHT in short term culture**

As the steroid hormone receptor expression levels were different between the endometrium and matched FT, with AR expression displaying the most striking difference, we investigated the effect of androgen (DHT) and E2 (which is the hormone with the most potent effects on the endometrium) on the expression of AR in the FT and matched endometrial samples. Downstream targets of E2, working via ERα (both *PR* and *AR* genes) and DHT working via AR (*AR* gene), were chosen to assess the hormone responsiveness of tubal and in endometrial tissue in a short term *in vitro* explant culture model.

In premenopausal endometrial explants in short-term culture, treatment with E2 or DHT did not significantly change the *AR* mRNA levels or immunostaining (protein) level. However, in the tubal explants, both treatments appeared to increase AR mRNA relative expression levels and AR immunostaining scores after 24 hours of treatment, suggesting that both hormones may be able to upregulate AR gene/protein (Figure 4). However, these descriptive observations were not statistically significant.

# Discussion

This study describes the expression of steroid hormone receptors in the healthy human FT epithelium, in comparison with the matched healthy endometrial epithelium, in relation to both proliferative activity and their response to hormone treatment. To our knowledge, this is the first study to comprehensively describe the differential pattern of epithelial proliferation and its relationship with steroid hormone receptor expression in the human FT, in comparison to the endometrium, across the menstrual cycle.

The fimbrial and/or isthmic portions of the FT were selected for use in IHC, and for qPCR and explant cultures, the epithelial rich fimbrial end was exclusively used. We considered the possibility of regional differences throughout the tubal lumen, as reported in some previous literature ([Amso, Crow, and Shaw 1994](#_ENREF_1); [Khan and Heatley 1999](#_ENREF_18); [Hill CJ 2020](#_ENREF_12)). However, in keeping with some other published studies, we found no statistically significant difference in either PI-Ki67 or steroid receptor immuno-scores between matched isthmic and fimbrial samples ([Christow, Sun, and Gemzell-Danielsson 2002](#_ENREF_4); [Sun et al. 2003](#_ENREF_36); [Pino et al. 1982](#_ENREF_30); [Punnonen and Lukola 1981](#_ENREF_31)). We have demonstrated that the tubal epithelium does not exhibit cyclical proliferation, in contrast to the matched endometrial epithelium, specifically the functional glands. This finding is consistent with the sparse amount of existing evidence ([Miyauchi et al. 2013](#_ENREF_25); [George, Milea, and Shaw 2012](#_ENREF_8); [Kuhn et al. 2012](#_ENREF_21)). We observed a dynamic spatial and temporal localisation of endometrial epithelial proliferative activity that was accompanied by corresponding cyclical changes in expression of steroid hormone receptors ERα, ERβ, PR and AR, consistent with the published literature ([Hapangama, Kamal, and Bulmer 2015](#_ENREF_10); [Critchley and Saunders 2009](#_ENREF_6)). The steroid hormone receptor expression profile of the FT epithelium was significantly different from the matched endometrial functional glandular epithelium*.* In contrast to the endometrial epithelium, FT epithelium did not display cyclical variation in ERα, ERβ, PR or AR. The most striking finding was the increased AR expression observed in the premenopausal FT epithelium when compared to the absent or very low AR immunostaining in the matched premenopausal endometrial glands. In a previous study, which only examined a limited number of FT (with no matched endometrium), it was shown that ERα, ERβ, and AR expression to be static across the menstrual cycle in the FT ([Horne et al. 2009](#_ENREF_13)). The relevance of the increased AR expression to the tubal epithelial proliferative activity is not known, but high epithelial AR immunoscores were also observed in the proliferatively quiescent PM endometrial epithelium ([Kamal, Bulmer, et al. 2016](#_ENREF_16)). Androgens levels have also been reported to correlate negatively with endometrial epithelial cellular proliferation ([Kamal, Bulmer, et al. 2016](#_ENREF_16)), and it has been shown that testosterone treatment is not associated with an increase in proliferation in the PM endometrium ([Zang et al. 2007](#_ENREF_42)). The endometrium and the tubal epithelium are both composed of columnar, ciliated and secretory cells, which form a barrier to the hostile ‘external’ environment and are exposed to the same circulating ovarian hormone levels. However, the intracellular levels of steroid hormone metabolising enzymes may differ, affecting the intracellular availability of the hormonal ligand at the cognate receptor, thus dictating the final functional impact of the hormone in the tissue. In agreement with this hypothesis, previous reports have demonstrated tubal epithelium to consistently express high levels of aldo-keto reductase family 1 member C3 (AKR1C3), an enzyme that activates androgen agonists testosterone and DHT ([Kuhn et al. 2013](#_ENREF_20)). Therefore, we postulate that local androgenic metabolism in the tubal epithelium favors androgenic milieu that harness the mitotic signal of the circulating oestrogens.

Our explant culture experiments using premenopausal tissue suggested that DHT treatment appears to increase *AR* mRNA and protein levels in the FT, an effect that was not observed in the endometrium. In the endometrium, *PR* is a downstream target of oestrogen ([Patel et al. 2015](#_ENREF_29)), where progesterone acts via PR to negate the E2 action ([Hapangama, Kamal, and Bulmer 2015](#_ENREF_10)). Interestingly, E2 did not increase tubal *PR* levels in our explant culture model, but short-term treatment with DHT appeared to increase both *PR* and *AR* levels. Considering the above data, we postulate that FT epithelium may be more responsive to androgens (and less responsive to E2) than the endometrium, and androgenic influence may maintain the low proliferative state in the tubal epithelium, which may be a prerequisite for normal FT function ([Kamal, Bulmer, et al. 2016](#_ENREF_16)), ([Russo, Young, and Burdette 2019](#_ENREF_32)). The primary function of the FT is to transport an embryo to the endometrium to facilitate the implantation of a normal intrauterine pregnancy ([Hunter 2012](#_ENREF_14)). Errors in this process can result in an ectopic pregnancy, which is often associated with FT pathology e.g. current or previous pelvic inflammatory disease (PID), or previous surgery ([Shaw et al. 2010](#_ENREF_35)), and studies have shown an increase in mucosal epithelial proliferation in FT pathology, for example salpingitis and ectopic pregnancy ([Yanai-Inbar and Silverberg 2000](#_ENREF_41)). Quiescent tubal epithelium thus may support normal tubal function. In our *in vitro* explant cultures, the endometrial samples were processed in whole, and the methodology did not allow deciphering of the region-specific or cell-specific differences in the hormone responsiveness ([Maclean et al. 2020](#_ENREF_23)). This is likely to be the reason for the modest changes we have observed in the gene expression. Further, mRNA expression does not always correlate with protein expression, and it is important to consider this when interpreting our qPCR and IHC data from *in vitro* tubal explant cultures.

Interestingly, proliferative indices in the tubal epithelium and AR immunoscores correlated positively with that of the matched endometrial LE. Endometrial LE is also reported to have a lower proliferative activity than the functional glands ([Wingfield et al. 1995](#_ENREF_40)). This data agrees with the notion that the LE of the endometrium exists as a continuum of the tubal epithelium and they may also have a common origin, thus be regenerated by a common progenitor cell ([Tempest et al. 2018](#_ENREF_37)).

This study contributes to the growing evidence for the existence of epithelial cells with functional differences and hormonal responsiveness in the human female reproductive tract ([Hapangama et al. 2019](#_ENREF_9)). Detailed knowledge and full characterization of the different types of epithelial cells in the human endometrium is currently lacking, and is an important area of research, as highlighted by the recent discovery of clonal growth of epithelium developing independently of stroma ([Noe et al. 2018](#_ENREF_27)), and the 3D-reconstruction of endometrium which revealed region-specific glandular microarchitecture ([Tempest et al. 2020](#_ENREF_38)).

Proliferative indices and steroid hormone receptor expression in the human FT is different to that of the matched human endometrium. These findings mark an important benchmark in the understanding of the normal human FT physiology. This study highlighted region specific differences of human endometrial epithelial cells and found some interesting similarities between the endometrial LE and FT epithelium. Further studies examining the differences in hormonal responsiveness and functional potential in both endometrial epithelial subtypes and in the tubal epithelium will pave the way to understanding many reproductive pathologies and to formulate targeted novel therapies for them.

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# Declaration of Author’s roles

D.K.H. obtained the ethical approval and conceived the study designs. The samples were collected by D.K.H., E.B., S.M., and A.M. D.K.H and A.J.V formulated experiments. Experiments were carried out and data collected by A.M., E.B., S.M., A.W., and A.K. A.M. and D.K.H analysed and interpreted data, produced figures, and produced the first draft. D.K.H and A.M. obtained funding. All authors revised the manuscript critically for intellectual content and approved the submitted version.

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**Disclosure/Conflict of Interest**

The authors declare that there is no conflict of interest.

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**Figure legends**

**Figure 1. *Percentage of cell staining of proliferative marker Ki67 in matched healthy human full-thickness endometrium and fallopian tube.*** (A) Proliferative phase, (B) secretory phase, and (C) postmenopausal women. (BG = basalis glands, FG = functional glands, LE = luminal epithelium, Tube = fallopian tube).

**Figure 2**. ***Representative high power (x 40) photomicropraphs illustrating the distribution of immunoreactivity (brown positive nuclear staining) for Ki67+, Androgen Receptor (AR) and Progesterone Receptor (PR) in the matched full-thickness normal human endometrial and fallopian tube samples across the menstrual cycle and in postmenopausal women***. Scale bars = 50 µM in all panels. PP = proliferative phase, SP = secretory phase, PM = postmenopausal women.

**Figure 3. *Immunoscores of steroid receptors in matched healthy human endometrial epithelium and fallopian tube (FT) epithelium.*** (A) Proliferative phase, (B) secretory phase, (C) postmenopausal women. Stacked graphs represent the mean expression of Androgen Receptor (AR), Progesterone Receptor (PR), Oestrogen receptors alpha (ERα) and beta (ERβ). (BG = basalis glands, FG = functional glands, LE = luminal epithelium, Tube = fallopian tube). The premenopausal tubal epithelial AR immunoscores were significantly higher when compared with the matched endometrial FG (6.7 ± 2.7 n=16 versus 0.3 ±1.0 n=10, *P*=0.013) and this was persistently significant when PP and SP samples were considered separately (PP, FT 5.1 ± 3.4, n=7 versus FG 0 ± 0, n=4, *P*=0.031) and (SP, FT 7.9 ± 1.3, n=9 versus FG 0.5 ±1.2, n=6, *P*=0.001), respectively.

**Figure 4**.

***Effect of oestradiol (E2) and dihydrotestosterone (DHT) treatment for 24 hours on AR mRNA levels and immunohistochemical staining in explant cultures of human endometrium and matched fimbrial end of the fallopian tube***. (A)– Endometrial *AR* mRNA expression after E2 treatment. (B)Tubal *AR* mRNA expression after E2 treatment. (C) – Endometrial *AR* mRNA expression after DHT treatment. (D) – Tubal *AR* mRNA expression after DHT treatment. (E) - Representative photomicrographs of tubal and endometrial explant immunohistochemical staining. Positive nuclear staining appears brown. Magnification x 400. Scale bars = 50 µM in all panels.

**Supplementary Figure 1:** Representative high power (x 40) photomicrographsillustrating the distribution of immunoreactivity (brown positive nuclear staining) for oestrogen receptors α (ERα) and β (ERβ) in the matched full-thickness normal human endometrial and matched fallopian tube samples across the menstrual cycle and in postmenopausal women. Scale bars = 50 µM in all panels. PP = proliferative phase, SP = secretory phase, PM = postmenopausal women.

**Supplementary Figure 2:** AR and PR mRNA expression in healthy premenopausal fimbrial end of the fallopian tube (FT) epithelium (A) *AR* (B) *PR*. (PP = proliferative phase, SP = secretory phase). No significant difference was observed in the samples.

**Supplementary Figure 3:** Correlation of AR and PR mRNA expression in (A) healthy premenopausal human endometrium (r=0.739, *P*=0.0003) and (B) healthy premenopausal fallopian tube (r=0.647, *P*=0.0027) from across the menstrual cycle and in postmenopausal state. (AR = androgen receptor, PR = progesterone receptor).

**Supplementary Figure 4:** AR immunoscores in healthy premenopausal human endometrium and fallopian tubes (FT). (A) endometrium (B) FT. \**P*<0.05, \*\**P*<0.001, \*\*\**P*<0.0001. (AR = androgen receptor). The AR immuno-staining was dominant in stromal compartment of the endometrium, while the tubal epithelium had much stronger staining than the stroma.