Journal of *Xenobiotics*

MDPI

Article

Effects of Sex Hormones and Physical Environment on Epithelial Cell Identity and Plasticity in an Engineered Urethral Model

Maxime Chaumery [1,†], Yudaï Sahuc [2,†], Stéphane Chabaud [2], Eric Philippe [3] and Stéphane Bolduc [2,4,*]

[1] Institut Universitaire de Technologie Génie Biologique, Université de Toulon, 83130 La Garde, France
[2] Centre de Recherche en Organogénèse Expérimentale/LOEX, CHU de Québec-Université Laval Research Center (Regenerative Medicine Division), Université Laval, Quebec City, QC G1J 4A4, Canada; yudai.sahuc@crchudequebec.ulaval.ca (Y.S.); stephane.chabaud@crchudequebec.ulaval.ca (S.C.)
[3] Laboratoire d'Anatomie, Surgery Department, Faculté de Médecine, l'Université Laval, Quebec City, QC G1V 0A6, Canada; eric.philippe@fmed.ulaval.ca
[4] Department of Surgery, Université Laval, Quebec City, QC G1V 0A6, Canada
* Correspondence: stephane.bolduc@fmed.ulaval.ca
† These authors contributed equally to this work.

Abstract

Cell identity within the human urogenital tract is strongly influenced by hormonal signaling, yet increasing evidence suggests that epithelial differentiation may also be shaped by microenvironmental cues. In this study, we investigated how hormonal and physical environments modulate epithelial identity and plasticity using a human tissue-engineered urethral model. Primary epithelial and stromal cells derived from vaginal and trans-women's urethral tissues were combined in homologous and heterologous configurations and cultured under controlled conditions. Constructs were exposed to estradiol or dihydrotestosterone, and epithelial differentiation was assessed using histological staining and immunofluorescence targeting lineage-specific markers. Our results show that vaginal epithelial cells cultured on vaginal stroma maintain a stable vaginal phenotype characterized by epithelial stratification and glycogen accumulation, although the magnitude of these features was modulated by hormonal stimulation. In contrast, urethral epithelial cells displayed a high degree of plasticity, adopting distinct phenotypes depending on the physical-culture environment. Notably, constructs containing urethral epithelium developed a dual phenotype, with vaginal-like differentiation at the air–liquid interface and a more urethral-like organization in immersed conditions. Furthermore, urethral cells derived from long-term estrogen-exposed tissues exhibited persistent expression of both urethral and vaginal markers, suggesting incomplete or hybrid differentiation states. Overall, these findings demonstrate that epithelial identity in the human urethra is not fixed but dynamically regulated by an interplay between intrinsic cellular properties, stromal interactions, and physical microenvironmental cues, while hormonal signaling alone appears insufficient to dictate cell fate. This study provides new insights into epithelial plasticity and has important implications for both developmental biology and tissue-engineering approaches to urogenital reconstruction.

Keywords: epithelial cell; differentiation; urethra; vagina; tissue engineering

Check for updates

Academic Editor: Yves Combarnous

Received: 31 August 2026
Revised: 23 October 2026
Accepted: 26 October 2026
Published: 1 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Human populations are increasingly exposed to endocrine disruptors (EDs), including chemical compounds commonly found in plastics (e.g., phthalates and bisphenols) and cosmetic products (e.g., methylparaben and benzylparaben) [1,2]. These compounds have the ability to interfere with the endocrine system by mimicking endogenous hormones such

as estrogens or by antagonizing androgen receptors [3,4]. Because sexual differentiation and organogenesis are tightly regulated by hormonal signaling, particularly androgens, such disruptions may significantly alter developmental processes [5,6].

Penile organogenesis is highly dependent on androgen signaling [7], and its perturbation can result in congenital malformations. Among these, hypospadias is one of the most common abnormalities of the male genitourinary tract, affecting approximately one in 150 live male births [8]. It is characterized by an ectopic positioning of the urethral meatus along the ventral aspect of the penis, with severity ranging from distal displacement to proximal forms near the scrotum [9,10]. This condition is associated with functional complications, including impaired urinary flow, penile curvature during erection [11], and an increased risk of subfertility.

Although genetic predisposition may contribute to hypospadias, epidemiological data indicate a worldwide increase in its incidence, strongly correlated with environmental changes and exposure to endocrine-disrupting compounds [1]. In particular, prenatal exposure to EDs has been shown to critically interfere with fetal development and to increase the risk of such malformations [12].

While epithelial tissues have traditionally been viewed as relatively stable once differentiated, increasing evidence indicates that differentiated epithelial cells may retain a degree of plasticity and responsiveness to environmental cues [13–15]. Cell identity is therefore not solely determined by developmental origin but may also be influenced by hormonal, stromal, and physical microenvironments [16,17].

During human urogenital development, the early urethral epithelium arises from a developmental context that is initially shared with the future vaginal epithelium [18–20]. The formation of the penile urethra involves canalization of the urethral plate followed by androgen-dependent distal fusion [21]. While the majority of the urethral epithelium is thought to derive from endoderm, the distal segment, known as the Fossa Navicularis, exhibits a particularly distinctive epithelial phenotype. This region contains a non-keratinized stratified squamous epithelium rich in glycogen, closely resembling vaginal epithelium and likely influenced by estrogen signaling [22,23]. These observations support the concept that urethral and vaginal epithelia may not represent completely distinct biological entities but rather different differentiation states along a shared developmental continuum. Such a model implies the existence of epithelial plasticity, whereby environmental signals may influence the acquisition or maintenance of tissue-specific characteristics.

From a clinical and tissue engineering perspective, the reconstruction of urethral tissues remains challenging due to the limited availability of suitable autologous material. To address this issue, a human urethral model reconstructed in vitro using the self-assembly method has been developed [24,25]. This approach enables the generation of stromal and epithelial compartments from primary human cells and provides a physiologically relevant platform to investigate tissue differentiation.

Recent advances have also identified gender-affirming surgeries as a novel and valuable source of human tissues for research. During vaginoplasty procedures, a significant portion of the urethra is surgically removed and can serve as a source of both urethral epithelial and stromal cells [26]. However, clinical and histological observations have reported the presence of vaginal-like epithelial features in these tissues, raising a broader biological question: are these changes solely the consequence of hormonal signaling, or do they reveal an intrinsic plasticity of urethral epithelial cells that can be modulated by their microenvironment?

One potential explanation is the long-term exposure to exogenous estrogens prior to surgery, typically administered for at least two years. Such hormonal treatments may influence cell fate and promote a partial shift toward a vaginal-like phenotype, as suggested

by earlier studies describing epithelial hyperplasia and increased glycogen accumulation within the spongy urethra under estrogenic conditions [22,23]. These findings highlight the potential plasticity of epithelial cells and the influence of the hormonal microenvironment on tissue identity.

In this context, the present study was designed to investigate epithelial plasticity within the human urogenital tract and to determine how hormonal, stromal, and physical environmental cues contribute to epithelial identity. Using a tissue-engineered urethral model, we combined vaginal and urethral epithelial cells with homologous and heterologous stromas to assess the relative contributions of epithelial origin and stromal context. Constructs were exposed to estradiol or dihydrotestosterone and analyzed using histological and molecular markers of urethral and vaginal differentiation. We hypothesized that epithelial identity is not exclusively dictated by cell origin or hormonal signaling but may emerge from dynamic interactions between epithelial cells, stromal cells, and the physical microenvironment.

2. Materials and Methods

2.1. Ethics Statement

This study was conducted according to the Declaration of Helsinki and was approved by the institution's committee for the protection of human participants (Comité d'éthique de la recherche du CHU de Québec-Université Laval, protocol number 2012-1341). All patients provided informed written consent prior to biopsies.

2.2. Sample Collection

A vaginal mucosal biopsy was obtained from a patient undergoing surgery for a benign condition of the female reproductive system. For this study, vaginal fibroblasts (VaFs) and vaginal epithelial cells (VaEs) were isolated from the vaginal mucosal biopsy of a 32-year-old woman [27].

Biopsies from the spongy urethra were obtained from 4 specimens undergoing gender-affirming vaginoplasty. For this study, urethral fibroblasts (UFs) and urethral epithelial cells (UEs) were isolated from urethral biopsies from female donors aged 23, 32, 38, and 51 [26].

2.3. Cell Culture

The fibroblasts (UF and VaF) were thawed and seeded at a density of 1.333×10^4 cells/cm^2 in 75-cm^2 culture flasks in Dulbecco–Vogt modified Eagle's medium (DMEMc, Corning, Corning, NY, USA), supplemented with 10% bovine calf serum (BCS, Woodland, CA, USA), 100 IU/mL penicillin (Sigma-Aldrich, St. Louis, MO, USA), and 25 µg/mL gentamicin (Schering, Pointe-Claire, QC, Canada) (Fb medium).

Epithelial cells (UE and VaE) were seeded at a density of 1.333×10^4 cells/cm^2 onto a feeder layer composed of irradiated foreskin fibroblasts and maintained in a medium consisting of a 3:1 mixture of DMEM and Ham's F12 (DHc) (Gibco, Grand Island, NY, USA). This medium was supplemented with 5% fetal bovine serum (FBS (Fetal Clone II), Citiva Hyclone, Logan, UT, USA), 24.3 µg/mL adenine (Corning), 5 µg/mL crystallized bovine insulin (Sigma-Aldrich, St. Louis, MO, USA), 1.1 µM hydrocortisone (Teva Canada Ltd., Scarborough, ON, Canada), 0.212 µg/mL isoproterenol hydrochloride (Sandoz, Boucherville, QC, Canada), 10 ng/mL epidermal growth factor (EGF, Austral Biologicals, San Ramon, CA, USA), 100 U/mL penicillin, and 25 µg/mL gentamicin (Epi medium).

2.4. Collagen Gel Production

A volume of 3.5 mL of a suspension of UF or VaF at 0.5 million cells per mL was mixed with 3.5 mL of cold collagen (5 mg/mL, PureCol EZ gel solution, Sigma Aldrich, St. Louis,

MO, USA) in ice until a homogenous solution was obtained. Sixty-six 1-mL collagen gels were generated for the study ($n = 3$ for each condition).

After gel solidification, metal supports were put in place to prevent them from contracting and 1 mL of Fb medium was added onto each gel. Media were changed three times a week. After one-week, UEs or VaEs at a density of 50,000 cells by cm^2 were seeded onto the gels in 1 mL of Epi medium. One week after seeding, the gels were transferred into 6-well plates and raised at the air/liquid interface, using metal supports, to allow differentiation of the epithelial cells. Three weeks later, the gels were ready for use.

During the whole process the cell-culture medium needed to contain 50 µg/mL of 2-phospho-L ascorbic acid (Sigma Aldrich, St. Louis, MO, USA) [19].

2.5. Hormonal Impact

To assess the impact of hormones, DHT (2 µM, Supelco D-073, Cerilliant, Round Rock, TX, USA) and E2 (10 ng/mL, Beta-estradiol, Sigma, St. Louis, MO, USA) were added to the cell-culture medium starting from the addition of epithelial cells onto the stroma and until the end of the experiment. For the control, no hormones were added in the cell-culture medium.

2.6. Histology and PAS Colorations

Half of each reconstructed tissue was fixed in 3.7% formalin, embedded in paraffin, sectioned at 6 µm, and stained using Masson's trichrome. Glycogen accumulation was assessed using periodic acid-Schiff staining (PAS) with or without prior diastase digestion (PASd). Briefly, paraffin sections were deparaffinized, rehydrated, subjected to diastase digestion when indicated, stained with periodic acid and Schiff reagent, counterstained with Harris hematoxylin, dehydrated, and mounted. Glycogen-positive structures appeared magenta, whereas PAS signal attributable to glycogen was abolished by diastase treatment.

2.7. Immunofluorescence Staining

Frozen OCT-embedded tissues were sectioned at 6 µm, fixed in cold acetone, and processed for immunofluorescence. Sections were incubated with primary antibodies against MUC1, ZO-1, K18, and K10 (Table 1), followed by fluorophore-conjugated secondary antibodies. Negative controls were incubated with PBS-BSA in the absence of primary antibody. Sections were mounted and analyzed by fluorescence microscopy. All original figures could be found in Supplementary Materials File S1.

Table 1. Antibodies used for immunofluorescence staining.

Antibody	Host Species and Specificity	Antigen and Conjugated Fluorochrome	Company	Dilution
Primary	Mouse; anti-human	Mucin-1	Santa Cruz	1/100
	Rabbit; anti-human	Zonula Occludens (ZO)-1	LSBio	1/50
	Mouse; anti-human	Keratin 18	ARP	1/100
	Guinea pig; anti-human	Keratin 10	Abcam	1/100
Secondary	Donkey; anti-rabbit	IgG Alexa 594	Life Technologies	1/200
	Goat; anti-mouse	IgG Alexa 488	Invitrogen	1/200
	Goat; anti-rabbit	IgG Alexa 594	Life Technologies	1/200
	Goat; anti-guinea pig	IgG Alexa 555	Life Technologies	1/200

3. Results

Histological observation of urethral mucosa from cis men and trans women indicated the presence of areas showing similarity to the vaginal epithelium; such was observed in the Fossa Navicularis of the urethra but clearly distinct from the neighboring spongy urethral mucosa of the one of the cis men (Figure 1).

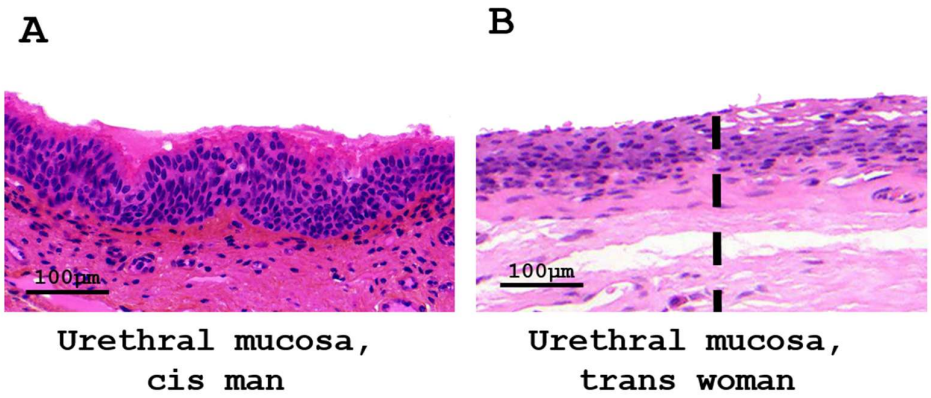


Figure 1. Histological comparison of native urethral mucosa from cisgender men and transgender women. Representative histological sections (Haematoxylin–Eosin) showing the epithelial organization of human urethral mucosa obtained from cisgender-male donors (**A**) and transgender women undergoing gender-affirming surgery (**B**). While both tissues displayed generally a similar epithelium, regions showing a vaginal-like morphology were observed in urethral tissues from transgender women. The dashed line indicates the approximate transition between urethral-like and vaginal-like epithelial regions.

To investigate the putative role of the origin of the stroma on the maturation of the urethral epithelium, epithelial cells from urethra (UEs) were seeded on their own stroma (UFs) or on the stroma produced using vaginal fibroblasts (VaFs). Alternatively, epithelial cells from vagina (VaEs) were seeded on their own stroma or on the stroma produced using urethral fibroblasts (Figure 2).

The UE+UF or UE+VaF conditions presented two phenotypes: urethral (Figure 2A1,B1), vaginal (Figure 2A2,B2), whereas VaE were unable to form an epithelium on UF-derived stromas (Figure 2C1,C2) and showed a well-differentiated vaginal epithelium in the VaE+VaF conditions (Figure 2D1,D2). Surprisingly, some sections of the constructs in the UE+UF or UE+VaF conditions presented a double epithelium with a vaginal-like morphology at the air/liquid faces and a urethral morphology at the submerged faces (Figure 2A3,B3).

A hallmark of the vaginal epithelium maturation is the presence of glycogenated cells. A Periodic Acid-Schiff staining (PAS) allows the detection of the polysaccharides, including the glycogen. The presence of the glycogen is specifically assessed by a subsequent treatment using the diastase which degrades the glycogen only (PASd).

When UE+UF and VaE+VaF were treated with PAS/PASd, presence of glycogen was detected in the UE+UF showing a urethral morphology (Figure 3A,B), in the UE+UF showing a vaginal morphology (Figure 3C,D), and in the control VaE+VaF (Figure 3E,F) indicating that even if the urethral mucosa reconstructed with transwomen cells presented a urethral morphology, a part of a vaginal-like phenotype is retained.

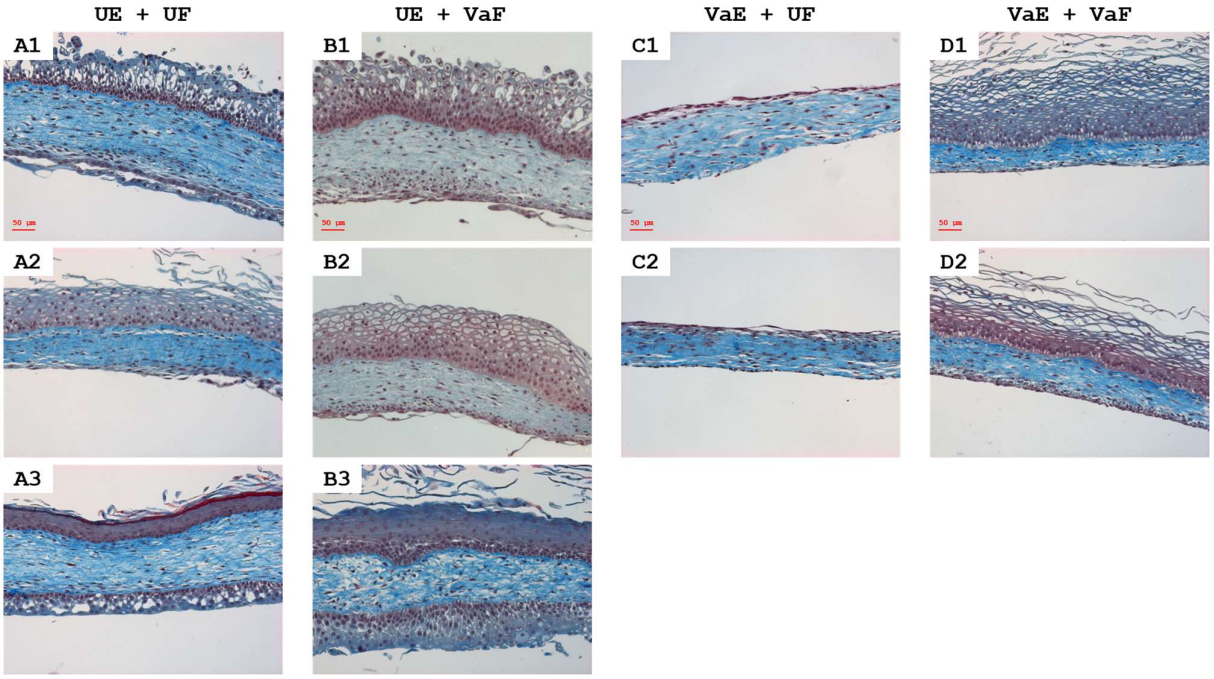


Figure 2. Effect of epithelial and stromal origin on tissue-engineered urethral reconstruction. Representative Masson's trichrome-stained sections of reconstructed tissues generated using homologous and heterologous combinations of urethral epithelial cells (UE), vaginal epithelial cells (VaE), urethral fibroblasts (UF), and vaginal fibroblasts (VaF). Tissue architecture, epithelial stratification, and stromal organization varied according to the epithelial-stromal pairing. (**A**) UE+UF tissue slices stained by Masson's trichrome. Representative examples included a urethral-like epithelium (**A1**), a vaginal-like epithelium (**A2**), and a double-sided epithelium (**A3**); (**B**) UE+VaF tissue slices stained by Masson's trichrome. Representative examples included a urethral-like epithelium (**B1**), a vaginal-like epithelium (**B2**), and a double-sided epithelium (**B3**); (**C**) VaE+UF tissue slices stained by Masson's trichrome. Representative examples showed an underdeveloped epithelium (**C1**,**C2**); (**D**) VaE+VaF tissue slices stained by Masson's trichrome. Representative examples showed a well-differentiated vaginal epithelium (**D1**,**D2**). (The blue staining corresponds to the collagens whereas the pink/purple staining represents cells). Scale bars = 50 µm.

To investigate the role of the hormones, dihydrotestosterone (DHT) and beta-estradiol (E2) were added to the cultures during the reconstruction of the tissues (Figure 4).

As shown previously, UE+UF without treatment (control) presented a urethral (Figure 4A1) or vaginal (Figure 4A2) epithelial morphology. Neither the DHT treatment (Figure 4B1,B2) nor the E2 treatment (Figure 4C1,C2) allowed any changes in the presentation of both morphologies. In the case of UE+VaF, contrarily to the results obtained with the control (Figure 4D1,D2), treatment with DHT abolished the ability of epithelial cells to proliferate and differentiate (Figure 4E1,E2) whereas E2 treatment seemed to have no effect (Figure 4F1,F2). Finally, compared with the vaginal epithelium obtained without treatment in the VaE+VaF conditions (Figure 4G1,G2), DHT seemed to induce a delay in maturation with more intermediate and less superficial (glycogenated) cells (Figure 4H1,H2) indicating that the hormones were efficient.

As indicated by Figures 2 and 4, in the case of epithelium present on both sides of the stroma, exposition to a submerged condition induced a urethral-like phenotype whereas the air/liquid interface induced a vaginal-like phenotype. These observations were done on several patients 23 y.o. (Figure 5A), 32 y.o. (Figure 5B), 38 y.o. (Figure 5C) and 51 y.o. (Figure 5D), indicating that it was not a patient-specific artefact but a reproducible result obtained in various donors of different ages.

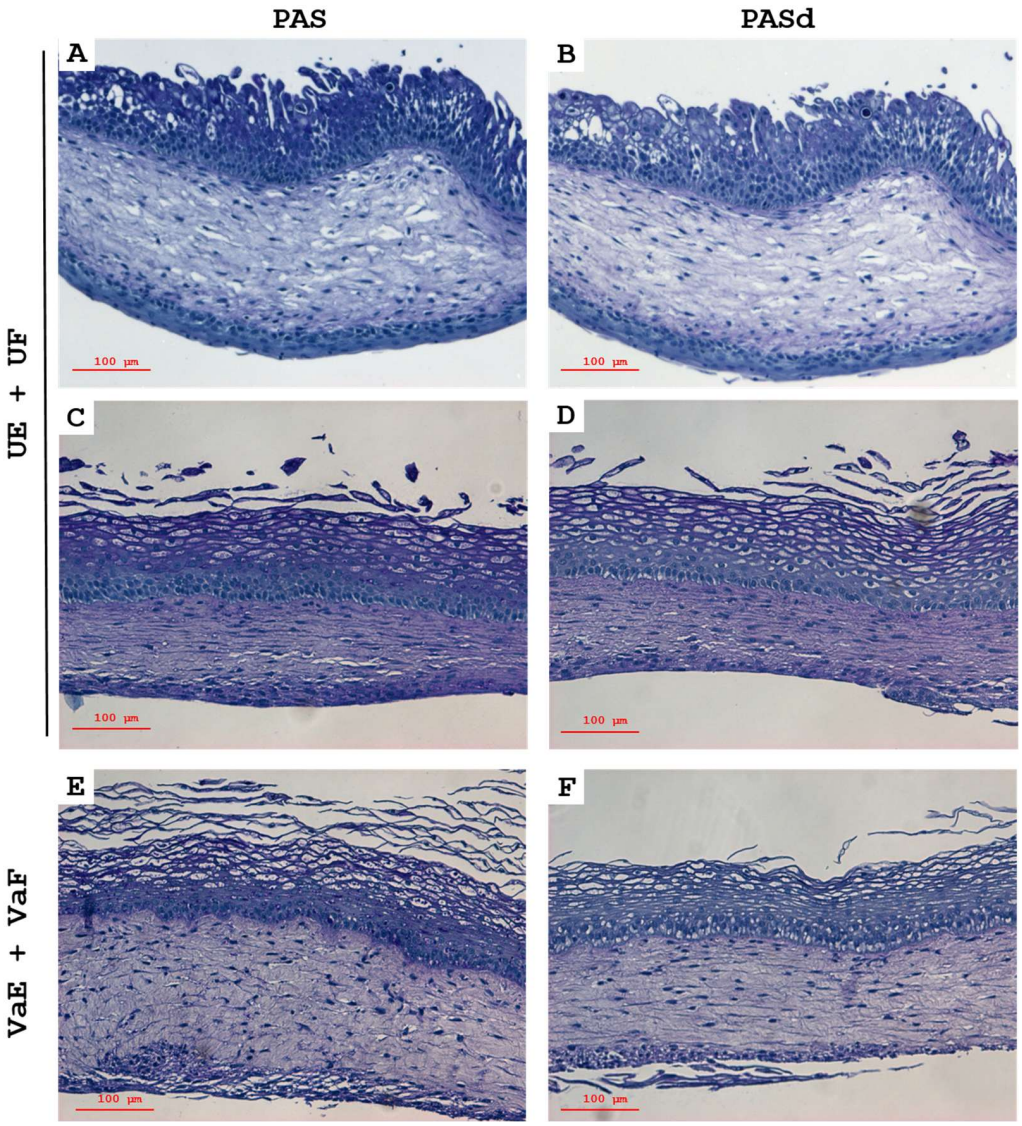


Figure 3. Glycogen accumulation in reconstructed tissues revealed by PAS staining. Representative Periodic Acid-Schiff (PAS) and PAS-diastase (PASd) staining performed on reconstructed tissues containing urethral or vaginal epithelial cells. PAS-positive regions correspond to the presence of polysaccharides. (**A**) UE+UF tissue slice stained by PAS staining; (**B**) UE+UF tissue slice stained by PAS staining in the presence of diastase—same tissue as in A panel; (**C**) another UE+UF tissue slice stained by PAS staining; (**D**) UE+UF tissue slice stained by PAS staining in the presence of diastase—same tissue as in C panel; (**E**) VaE+VaF tissue slice stained by PAS staining; (**F**) VaE+VaF tissue slice stained by PAS staining in the presence of diastase—same tissue as in E panel. PAS-Positive staining was largely abolished following diastase treatment, confirming glycogen accumulation within epithelial cells. (Counterstaining was done with haematoxylin-eosin). Scale bars = 100 µm.

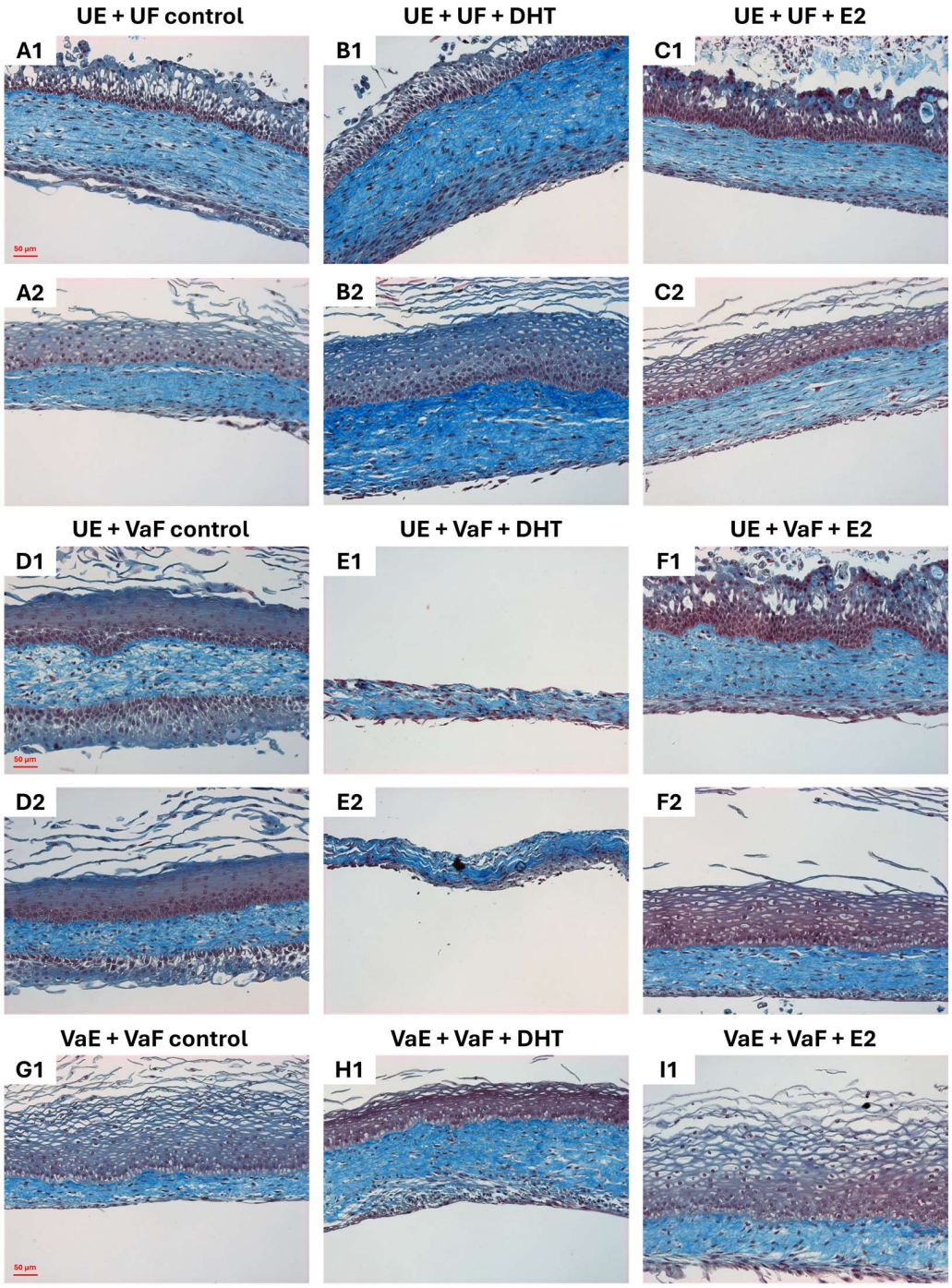


Figure 4. *Cont.*

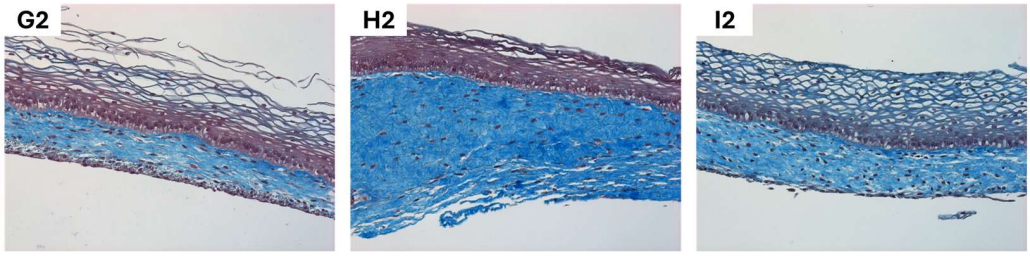


Figure 4. Impact of hormonal stimulation on epithelial differentiation. Representative Masson's trichrome-stained sections of reconstructed tissues cultured under control conditions or exposed to estradiol (E2) or dihydrotestosterone (DHT). Histological analysis revealed that vaginal epithelial cells on vaginal stroma retained a stable vaginal phenotype under all conditions, whereas urethral epithelial cells exhibited limited morphological changes in response to hormonal stimulation. Panels illustrate representative sections for each epithelial-stromal combination. (**A**–**C**) Masson's trichrome-stained sections of UE+UF tissues: (**A**) Control (representative examples included (**A1**): urethral-like epithelium, (**A2**): vaginal-like epithelium); (**B**) +DHT (representative examples included (**B1**): urethral-like epithelium, (**B2**): vaginal-like epithelium); and (**C**) +E2 (representative examples included (**C1**): urethral-like epithelium, (**C2**): vaginal-like epithelium); (**D**–**F**) Masson's trichrome-stained sections of UE+VaF tissues: (**D**) Control, (**D1**,**D2**) are representative examples of double-sided epitheliums; (**E**) +DHT (**E1**,**E2**) are representative examples of underdeveloped epitheliums); and (**F**) +E2 (representative examples included (**F1**): urethral-like epithelium, (**F2**): vaginal-like epithelium); (**G**–**I**) Masson's trichrome-stained sections of VaE+VaF tissues: (**G**) Control (**G1,G2**) are representative examples of control epitheliums); (**H**) +DHT (**H1**,**H2**) are representative examples of thinner epitheliums); and (**I**) +E2 (**I1**,**I2**) are representative examples of thicker epitheliums). (The blue staining correspond to the collagens whereas the pink/purple staining represents cells). Scale bars = 50 μm.

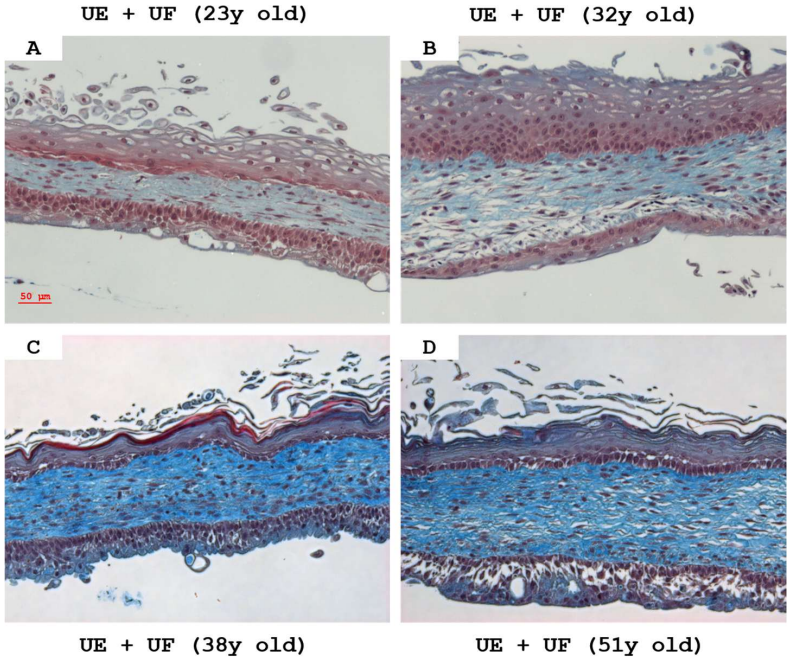


Figure 5. Reproducibility of the urethral epithelial phenotype among independent donors. Representative Masson's trichrome-stained sections of engineered tissues reconstructed using urethral epithelial and stromal cells isolated from four transgender donors aged 23, 32, 38, and 51 years. Similar epithelial organization was observed across donors, supporting the reproducibility of the observed phenotype: representative example of UE+UF tissue slice stained by Masson's trichrome from: (**A**) a 23-year-old donor; (**B**) a 32-year-old donor; (**C**) a 38-year-old donor; and (**D**) a 51-year-old donor. (The blue staining correspond to the collagens whereas the pink/purple staining represents cells). Scale bars = 50 μm.

The plasticity of the epithelial cells from urethras of transgender women, and the ability to adopt urethral or vaginal morphologies was further investigated using a molecular marker of the vaginal epithelium (Mucin-1 and K10). Expression of MUC1 and K10 was detected in UE+UF (Figure 6A1,A2) and UE+VaF (Figure 6B1,B2) constructs as in the pure vaginal control (Figure 6C1,C2).

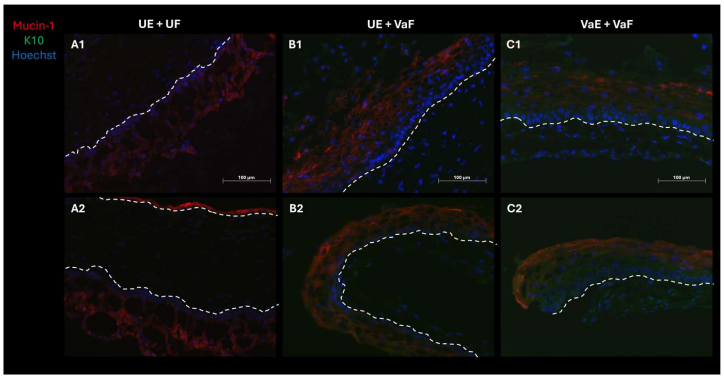


Figure 6. Immunofluorescent analysis of vaginal differentiation markers. Representative immunofluorescence (IF) staining of reconstructed tissues for MUC1 (red) and keratin 10 (K10, green). Cell nuclei were counterstained with Hoechst (blue). Dashed line corresponds to the boundary between epithelium and stroma. (**A**) IF on UE+UF tissue slices with representative examples shown in (**A1**,**A2**); (**B**) IF on UE+VaF tissue slices with representative examples shown in (**B1**,**B2**); (**C**) IF on VaE+VaF tissue slices with representative examples shown in (**C1**,**C2**). Scale bars = 100 µm.

The expression of urethral markers (ZO-1 and K18) was also monitored in the UE-containing constructs. Both types of construction UE+UF (Figure 7A1,A2) and UE+VaF (Figure 7B1,B2) express urethral markers indicating that they were able to express not only urethral markers but also vaginal markers (Figure 6).

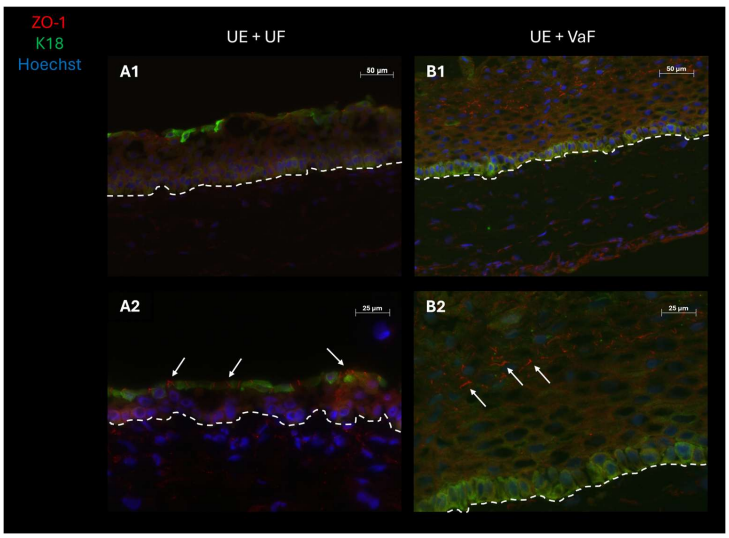


Figure 7. Immunofluorescent analysis of urethral differentiation markers. Representative immunofluorescence staining for zonula occludens-1 (ZO-1, red) and keratin 18 (K18, green) in reconstructed tissues. Cell nuclei were counterstained with Hoechst (blue). Expression of urethral-associated markers was maintained in tissues containing urethral epithelial cells, despite the acquisition of vaginal-like morphological characteristics in specific microenvironmental conditions. Arrows indicate areas showing apical localization of ZO-1. Dashed line corresponds to the boundary between epithelium and stroma. (**A**) IF on UE+UF tissue slices with representative examples shown in (**A1**,**A2**); (**B**) IF on UE+VaF tissue slices with representative examples shown in (**B1**,**B2**). Scale bars = 50 µm (**A1**,**B1**) and 25 µm (**A2**,**B2**).

4. Discussion

The present study highlights the remarkable stability of vaginal epithelial identity under varying hormonal conditions. Constructs generated from vaginal fibroblasts and epithelial cells (VaFs/VaEs) consistently exhibited a well-differentiated stratified epithelium with glycogen accumulation, typical of vaginal tissue architecture (Figure 2D1,D2), Figure 4G1,G2). Although exposure to DHT led to a delay in maturation and an increase in the number of intermediate cell layers (Figure 4H1,H2) and exposure to E2 resulted in a moderate increase in epithelial thickness (Figure 4I1,I2), the overall phenotype remained unchanged. This robustness suggests that vaginal epithelial identity is strongly preconditioned by both epithelial and stromal compartments according to the literature [28–30]. The maintenance of Mucin-1 + K10 expression further confirm the persistence of a vaginal differentiation program (Figure 6C1,C2). These findings support the concept that, once established, vaginal epithelial identity may be relatively resistant to short-term hormonal perturbations in vitro, likely due to stable stromal–epithelial signaling loops.

One of the most striking observations in this study is the complete absence of epithelial formation in heterologous constructs combining urethral fibroblasts with vaginal epithelial cells (UFs/VaEs) (Figure 2C1,C2). This result underscores the essential role of stromal support in epithelial survival and organization. Stromal cells are known to provide key biochemical and mechanical cues that regulate epithelial proliferation, differentiation, and polarity [31]. The inability of vaginal epithelial cells to establish or maintain an epithelial layer in the presence of urethral fibroblasts suggests a strong dependence on tissue-specific stromal signaling [27,31–33]. This incompatibility may reflect differences in extracellular matrix composition, paracrine signaling, or mechanical properties of the stroma. These findings reinforce the concept that epithelial identity cannot be considered independently of its stromal microenvironment, and that mismatch between these compartments may lead to epithelial instability or loss.

In contrast, constructs involving urethral epithelial cells (UEs), whether combined with urethral fibroblasts (UFs/UEs) or vaginal fibroblasts (VaFs/UEs), exhibited a highly reproducible and unexpected phenotype (Figure 2A1–A3,B1–B3). In both conditions, epithelial coverage was consistently observed on both surfaces of the construct, indicating a strong intrinsic capacity of urethral epithelial cells to reorganize under the given culture conditions. This behavior contrasts sharply with vaginal epithelial cells and suggests that urethral epithelial cells may possess a higher degree of plasticity or adaptability. Notably, stromal origin did not appear to be the dominant determinant of epithelial outcome in these constructs, further emphasizing the importance of epithelial intrinsic properties in this context. The reason why the urethral epithelial cells on a vaginal stroma seem unable to proliferate and differentiate in the presence of DHT remains to be determined.

A central finding of this study is the emergence of a dual epithelial phenotype depending on the physical culture environment. In constructs containing urethral epithelial cells, two distinct epithelial profiles developed: on the air–liquid interface, the epithelium exhibited a predominantly vaginal-like phenotype, characterized by stratification, glycogen accumulation, and expression of vaginal markers (K10, MUC1), while retaining partial urethral features such as luminal localization of ZO-1. On the immersed side, the epithelium displayed a morphology and organization more consistent with a urethral phenotype, although some vaginal markers were still detected (Figures 6 and 7).

The pronounced divergence in epithelial phenotypes observed between air–liquid interface and submerged conditions suggests that physical cues may play an important role in epithelial differentiation (Figure 8) [34,35]. Rather than relying on a single determinant, this divergence could result from a combination of interrelated microenvironmental factors, including oxygen availability, hydration state, and the diffusion of nutrients and growth

factors, which may generate distinct biochemical gradients across the tissues. Such gradients are known to influence epithelial metabolism, polarity, and barrier function and may therefore contribute to the establishment of divergent differentiation programs [35–41]. Under these conditions, the air–liquid interface appeared to favor stratification and glycogen accumulation (Figure 2A2,A3,B2,B3), recapitulating several characteristics of vaginal mucosa, whereas immersion was associated with a simpler, more urethral-like organization (Figure 2A1,A3,B1,B3). In addition, epithelial cells may actively modify their local microenvironment through the secretion of soluble factors capable of influencing stromal remodeling and epithelial differentiation [42,43]. Stromal mechanical properties could also contribute to these effects, although the relative importance of these mechanisms remains to be determined. Altogether, these observations support the existence of a remarkable epithelial plasticity, whereby a single cell population can adopt distinct differentiation trajectories probably through a complex interplay between physical constraints, biochemical gradients, and reciprocal stromal–epithelial interactions.

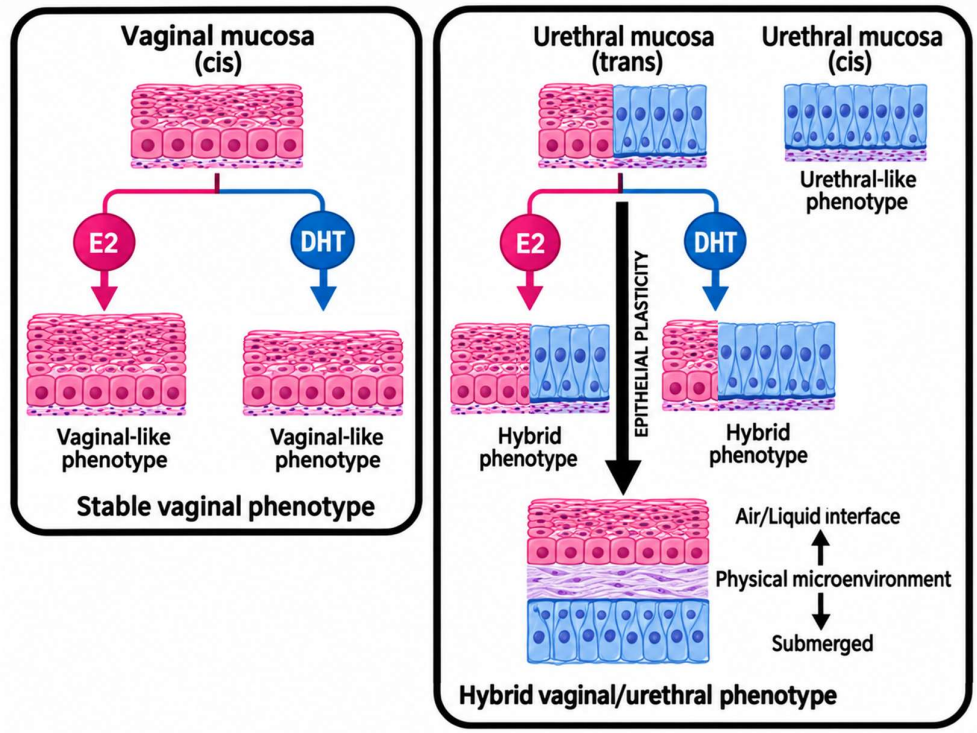


Figure 8. Proposed model of urethral epithelial plasticity. A common embryological origin may explain the ability of urethral epithelial cells to retain vaginal-like characteristics. While hormonal signaling modulates epithelial differentiation, urethral epithelial cells derived from transgender women exhibit a hybrid urethral/vaginal phenotype. Our findings suggest that the physical microenvironment, particularly the air–liquid interface versus submerged culture conditions, may represent an important determinant of the final epithelial phenotype.

While hormonal treatments (DHT and E2) were expected to play a major role in guiding tissue differentiation, their effects appeared secondary compared to those of the physical microenvironment and cellular context [44]. Although E2 influenced epithelial thickness in vaginal constructs (Figure 4I1,I2), it did not induce a phenotypic switch in urethral constructs (Figure 4C1,D1,D2,F1). Similarly, androgen exposure did not fully stabilize a urethral phenotype in urethral constructs (Figure 4B2) whereas it had a little impact on vaginal constructs (Figure 4H1,H2). These findings suggest that hormonal signaling alone, in the conditions tested, may be insufficient to dictate epithelial identity in vitro and must be interpreted within a broader framework that includes stromal interactions and physical

constraints. The partial persistence of vaginal markers in urethral-like epithelium further supports the idea of incomplete or reversible differentiation states.

The observed plasticity between urethral and vaginal phenotypes provides new insights into urogenital development. The coexistence of both phenotypes within the same construct supports the notion of a shared developmental origin and suggests that urethral epithelial cells may retain the capacity to reactivate differentiation programs associated with a more vaginal-like state. In this context, inappropriate exposure to endocrine disruptors or exogenous hormones during development could potentially influence epithelial differentiation and contribute to developmental abnormalities such as hypospadias. The persistence of vaginal-associated features in urethral cells derived from transgender women is consistent with the possibility that such latent differentiation programs may remain accessible beyond development and could be modulated by hormonal or microenvironmental cues later in life. However, because endocrine disruptors were not directly investigated in the present study, these interpretations should be considered as hypotheses requiring further experimental validation.

From a translational perspective, these findings have important implications for urethral reconstruction. The strong influence of the physical microenvironment suggests that culture conditions must be carefully controlled to achieve a stable and physiologically relevant epithelial phenotype.

Furthermore, the potential for vaginal-like differentiation in urethral constructs raises concerns regarding the selection of cell sources, particularly in contexts involving prior hormonal treatments, such as gender-affirming therapies. The persistence of mixed phenotypes may impact the functional properties of engineered tissues and should be considered in future applications.

This study has several limitations that should be acknowledged. First, the analysis was restricted to a defined set of histological and immunofluorescent markers, which, although informative, may not fully capture the complexity of epithelial identity. Second, the duration and conditions of hormonal treatments may not fully reproduce physiological exposure scenarios.

Future studies should aim to expand molecular characterization using transcriptomic or proteomic approaches, investigate the reversibility of observed phenotypes, explore the contribution of mechanical forces and oxygen gradients in greater detail, and assess functional properties of the reconstructed epithelia.

5. Conclusions

Overall, this study demonstrates that epithelial identity within the human urethra is not fixed but highly plastic, shaped by an interplay between stromal interactions, hormonal signaling, and physical microenvironment. These findings provide new insights into urogenital biology and highlight important considerations for both developmental biology and tissue-engineering applications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jox16070211/s1, File S1: Original Images.

Author Contributions: Conceptualization, Y.S. and S.C.; methodology, M.C., Y.S., and S.C.; validation, Y.S. and S.C.; formal analysis, M.C., Y.S., and S.C.; resources, E.P. and S.B.; data curation, Y.S. and S.C.; writing—original draft preparation, M.C., Y.S., and S.C.; writing—review and editing, M.C., Y.S., S.C., E.P., and S.B.; supervision, E.P. and S.B.; project administration, S.B.; funding acquisition, S.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by funding from the CIHR (258229 and 178186) and the New Frontiers in Research Fund (GF41743).

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the CHU de Québec, protocol code 2012-1341, DR-002-1190, date 29 May 2007 (conducted again each year from this date).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors wish to acknowledge the Quebec Cell, Tissue and Gene Therapy Network-ThéCell (a thematic network supported by the FRQS) and the Fonds de recherche du Québec-Santé (FRQS) through the research center grant for the CHU de Québec-Université Laval Research Center (reference: 30641). The authors declare that Generative AI (Copilot Microsoft 365, Microsoft Corp, Redmont, WA, USA) was used in the preparation of this manuscript solely to improve the language of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Caneparo, C.; Carignan, L.; Lonina, E.; Goulet, S.M.; Pellerin, F.A.; Chabaud, S.; Bordeleau, F.; Bolduc, S.; Pelletier, M. Impact of Endocrine Disruptors on the Genitourinary Tract. *J. Xenobiotics* **2024**, *14*, 1849–1888. [CrossRef] [PubMed]
2. Gracidas, C.; Selem, E.; Anyiam, N.D.; Fertig, B.; Varon, J.; Halma, M. Overview of the Most Common Endocrine Disruptors: Exposure, Mechanism, Health Effects, and Remediation Strategies. *Environ. Toxicol.* **2026**, *41*, 1323–1363. [CrossRef] [PubMed]
3. Monneret, C. What is an endocrine disruptor? *Comptes Rendus. Biol.* **2017**, *340*, 403–405. [CrossRef] [PubMed]
4. Yoon, K.; Kwack, S.J.; Kim, H.S.; Lee, B.M. Estrogenic endocrine-disrupting chemicals: Molecular mechanisms of actions on putative human diseases. *J. Toxicol. Environ. Health B Crit. Rev.* **2014**, *17*, 127–174. [CrossRef] [PubMed]
5. Baykal, R.G.; Ersoy, R. The effects of endocrine disruptors on the female reproductive system. *Turk. J. Med. Sci.* **2025**, *55*, 1641–1647. [CrossRef] [PubMed]
6. Baskin, L.; Shen, J.; Sinclair, A.; Cao, M.; Liu, X.; Liu, G.; Isaacson, D.; Overland, M.; Li, Y.; Cunha, G.R. Development of the human penis and clitoris. *Differentiation* **2018**, *103*, 74–85. [CrossRef] [PubMed]
7. Ray, R.; Racine, C. Sexual Differentiation. In *Endotext*; Feingold, K.R., Adler, R.A., Ahmed, S.F., Anawalt, B., Blackman, M.R., Chrousos, G., Corpas, E., de Herder, W.W., Dhatariya, K., Dungan, K., et al., Eds.; MDText.com, Inc.: Portland, OR, USA, 2000.
8. Lavoie, C.; Chun, B.; Au, M.; Do, C.; Baker, Z.; Cortessis, V.; Sparks, S.S.; Syed, H.; Chang, A.Y. Comparing the incidence of hypospadias across the United States: A contemporary analysis. *J. Pediatr. Urol.* **2025**, *21*, 627–632. [CrossRef] [PubMed]
9. Springer, A. Assessment of outcome in hypospadias surgery—A review. *Front. Pediatr.* **2014**, *2*, 2. [CrossRef] [PubMed]
10. Halaseh, S.A.; Halaseh, S.; Ashour, M. Hypospadias: A Comprehensive Review Including Its Embryology, Etiology and Surgical Techniques. *Cureus* **2022**, *14*, e27544. [CrossRef] [PubMed]
11. Snodgrass, W.; Bush, N. Recent advances in understanding/management of hypospadias. *F1000Prime Rep.* **2014**, *6*, 101. [CrossRef] [PubMed]
12. Bonde, J.P.; Flachs, E.M.; Rimborg, S.; Glazer, C.H.; Giwercman, A.; Ramlau-Hansen, C.H.; Hougaard, K.S.; Hoyer, B.B.; Haervig, K.K.; Petersen, S.B.; et al. The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: A systematic review and meta-analysis. *Hum. Reprod. Update* **2016**, *23*, 104–125. [CrossRef] [PubMed]
13. Blanpain, C.; Fuchs, E. Stem cell plasticity. Plasticity of epithelial stem cells in tissue regeneration. *Science* **2014**, *344*, 1242281. [CrossRef] [PubMed]
14. Tata, P.R.; Rajagopal, J. Plasticity in the lung: Making and breaking cell identity. *Development* **2017**, *144*, 755–766. [CrossRef] [PubMed]
15. Wu, K.; Liu, X.; Zhang, J.; Wang, X.; Li, X.; Chen, C. Mechanisms and implications of epithelial cell plasticity in the bladder. *Nat. Rev. Urol.* **2025**, *23*, 70–88. [CrossRef] [PubMed]
16. Nagai, H.; Nakajima, Y.I. Epithelial cell plasticity in metazoans: Evolutionary insights into roles and mechanisms. *Semin. Cell Dev. Biol.* **2026**, *179–180*, 103670. [CrossRef] [PubMed]
17. Tata, A.; Chow, R.D.; Tata, P.R. Epithelial cell plasticity: Breaking boundaries and changing landscapes. *EMBO Rep.* **2021**, *22*, e51921. [CrossRef] [PubMed]
18. Robboy, S.J.; Kurita, T.; Baskin, L.; Cunha, G.R. New insights into human female reproductive tract development. *Differentiation* **2017**, *97*, 9–22. [CrossRef] [PubMed]

19. Cunha, G.R.; Sinclair, A.; Risbridger, G.; Hutson, J.; Baskin, L.S. Current understanding of hypospadias: Relevance of animal models. *Nat. Rev. Urol.* **2015**, *12*, 271–280. [CrossRef] [PubMed]
20. Baskin, L.S.; Ebbers, M.B. Hypospadias: Anatomy, etiology, and technique. *J. Pediatr. Surg.* **2006**, *41*, 463–472. [CrossRef] [PubMed]
21. Cunha, G.R.; Baskin, L. Development of human male and female urogenital tracts. *Differentiation* **2018**, *103*, 1–4. [CrossRef] [PubMed]
22. Duval, M.; Brownell, D.; Chabaud, S.; Laungani, A.; Philippe, E.; Bolduc, S. Description of the Human Penile Urethra Epithelium. *Medicina* **2025**, *61*, 788. [CrossRef] [PubMed]
23. Holstein, A.F.; Davidoff, M.S.; Breucker, H.; Countouris, N.; Orlandini, G. Different epithelia in the distal human male urethra. *Cell Tissue Res.* **1991**, *264*, 23–32. [CrossRef] [PubMed]
24. Caneparo, C.; Chabaud, S.; Fradette, J.; Bolduc, S. Engineered human organ-specific urethra as a functional substitute. *Sci. Rep.* **2022**, *12*, 21346. [CrossRef] [PubMed]
25. Caneparo, C.; Elia, E.; Chabaud, S.; Berthod, F.; Fradette, J.; Bolduc, S. Correction of Significant Urethral Anomalies Using a Tissue-Engineered Human Urethral Substitute: Proof of Concept. *Int. J. Mol. Sci.* **2025**, *26*, 1825. [CrossRef] [PubMed]
26. Brownell, D.; Elia, E.; Pellerin, F.A.; Chabaud, S.; Larochelle, S.; Moulin, V.J.; Laungani, A.; Bolduc, S. Isolation and characterization of epithelial cells and fibroblasts from the human penile urethra. *Front. Bioeng. Biotechnol.* **2025**, *13*, 1713156. [CrossRef] [PubMed]
27. Saba, I.; Barat, C.; Chabaud, S.; Reyjon, N.; Leclerc, M.; Jakubowska, W.; Orabi, H.; Lachhab, A.; Pelletier, M.; Tremblay, M.J.; et al. Immunocompetent Human 3D Organ-Specific Hormone-Responding Vaginal Mucosa Model of HIV-1 Infection. *Tissue Eng. Part C Methods* **2021**, *27*, 152–166. [CrossRef] [PubMed]
28. Cunha, G.R. Stromal induction and specification of morphogenesis and cytodifferentiation of the epithelia of the Mullerian ducts and urogenital sinus during development of the uterus and vagina in mice. *J. Exp. Zool.* **1976**, *196*, 361–370. [CrossRef] [PubMed]
29. Cunha, G.R.; Cooke, P.S.; Kurita, T. Role of stromal-epithelial interactions in hormonal responses. *Arch. Histol. Cytol.* **2004**, *67*, 417–434. [CrossRef] [PubMed]
30. Kurita, T.; Cooke, P.S.; Cunha, G.R. Epithelial-stromal tissue interaction in paramesonephric (Mullerian) epithelial differentiation. *Dev. Biol.* **2001**, *240*, 194–211. [CrossRef] [PubMed]
31. Rousseau, A.; Fradette, J.; Bernard, G.; Gauvin, R.; Laterreur, V.; Bolduc, S. Adipose-derived stromal cells for the reconstruction of a human vesical equivalent. *J. Tissue Eng. Regen. Med.* **2015**, *9*, E135–E143. [CrossRef] [PubMed]
32. Rinn, J.L.; Bondre, C.; Gladstone, H.B.; Brown, P.O.; Chang, H.Y. Anatomic demarcation by positional variation in fibroblast gene expression programs. *PLoS Genet.* **2006**, *2*, e119. [CrossRef] [PubMed]
33. Carrier, P.; Deschambeault, A.; Audet, C.; Talbot, M.; Gauvin, R.; Giasson, C.J.; Auger, F.A.; Guerin, S.L.; Germain, L. Impact of cell source on human cornea reconstructed by tissue engineering. *Investig. Ophthalmol. Vis. Sci.* **2009**, *50*, 2645–2652. [CrossRef] [PubMed]
34. Fulcher, M.L.; Gabriel, S.; Burns, K.A.; Yankaskas, J.R.; Randell, S.H. Well-differentiated human airway epithelial cell cultures. In *Methods in Molecular Medicine*; Springer: Berlin/Heidelberg, Germany, 2005; Volume 107, pp. 183–206.
35. Pezzulo, A.A.; Starner, T.D.; Scheetz, T.E.; Traver, G.L.; Tilley, A.E.; Harvey, B.G.; Crystal, R.G.; McCray, P.B., Jr.; Zabner, J. The air-liquid interface and use of primary cell cultures are important to recapitulate the transcriptional profile of in vivo airway epithelia. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2011**, *300*, L25–L31. [CrossRef] [PubMed]
36. Colgan, S.P.; Taylor, C.T. Hypoxia: An alarm signal during intestinal inflammation. *Nat. Rev. Gastroenterol. Hepatol.* **2010**, *7*, 281–287. [CrossRef] [PubMed]
37. McKeown, S.R. Defining normoxia, physoxia and hypoxia in tumours-implications for treatment response. *Br. J. Radiol.* **2014**, *87*, 20130676. [CrossRef] [PubMed]
38. Rodriguez-Boulan, E.; Macara, I.G. Organization and execution of the epithelial polarity programme. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 225–242. [CrossRef] [PubMed]
39. Hild, M.; Jaffe, A.B. Production of 3-D Airway Organoids From Primary Human Airway Basal Cells and Their Use in High-Throughput Screening. *Curr. Protoc. Stem Cell Biol.* **2016**, *37*, IE 9 1–IE 9 15. [CrossRef] [PubMed]
40. Marchiando, A.M.; Graham, W.V.; Turner, J.R. Epithelial barriers in homeostasis and disease. *Annu. Rev. Pathol.* **2010**, *5*, 119–144. [CrossRef] [PubMed]
41. Discher, D.E.; Mooney, D.J.; Zandstra, P.W. Growth factors, matrices, and forces combine and control stem cells. *Science* **2009**, *324*, 1673–1677. [CrossRef] [PubMed]
42. Baskin, L.S.; Hayward, S.W.; Sutherland, R.A.; DiSandro, M.J.; Thomson, A.A.; Goodman, J.; Cunha, G.R. Mesenchymal-epithelial interactions in the bladder. *World J. Urol.* **1996**, *14*, 301–309. [CrossRef] [PubMed]

43. Mendelsohn, C. Going in circles: Conserved mechanisms control radial patterning in the urinary and digestive tracts. *J. Clin. Investig.* **2006**, *116*, 635–637. [CrossRef] [PubMed]
44. Elmelund, E.; Draskau, M.K.; Stewart, M.K.; Mattiske, D.M.; Pask, A.J.; Svingen, T. Revisiting the dual role of androgens and oestrogens in mammalian sex differentiation with a focus on genitalia. *Nat. Rev. Urol.* **2026**, *23*, 621–629. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.