

REVIEW

The development of the human vaginal fornix and the portio cervicis

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Abstract

Introduction: One of the transitional zones of the human body is situated in the cervix uteri. The developmental differentiation of epithelial and stromal characteristics in such a region is of high clinical interest. However, few studies have focused on the development of this region, and information in anatomical and clinical textbooks is limited. We therefore examined the development of the human vaginal fornix and the cervix uteri during prenatal development.

Materials and Methods: We examined 29 female embryos and fetuses between 20 and 34 weeks and two newborns using histology and immunohistochemistry.

Results: The characteristic shape of the portiocervicis and the vaginal fornix first became visible in mid-term fetuses because of the different muscular coats and of an uncategorized Müllerian-derived epithelium, which was rapidly replaced by a multilayered squamous epithelium. Only thereafter, in older fetuses, were there organogenetic differentiation of the epithelia and the underlying stroma of the cervical canal. UGS-derived p63/CK17-positive cells could be identified as precursor cells for the squamous epithelium, and Müllerian-derived CK7-positive cells for the columnar-type epithelium. Both cell types and different stromal zones were already present in a prenatal transformation zone. Initial functional differentiation could be observed in perinatal stages.

Conclusions: Our results on prenatal human development strongly support the view that two different cell lineages meet at the transitional zone of the cervix uteri and that these lineages depend on alternative signals from the underlying stromal compartment.

KEYWORDS

human prenatal development, mesenchymal stroma, portiocervicis, precursor cells, transformation zone, vaginal fornix

1 | INTRODUCTION

Apart from remarks about a common origin of the Müllerian ducts (MDs), standard textbooks of human embryology (Moore, Persaud, &

Torchia, 2016; Sadler, 2019) and anatomy (Standring, 2008 in Gray's Anatomy) contain no detailed information about the development of the cervix uteri or of the portiocervicis and vaginal fornix. This could be because these textbooks focus mainly on the connection between

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normal development, malformations and genetic disorders. However, malformations of the uterus, the cervix and the vagina are rare (Cunha et al., 2018). Nevertheless, new insights into the normal development of the portiocervix, which is subdivided into supravaginal (clinically endocervix) and vaginal (clinically ectocervix) portions, are of high clinical importance since the cervix belongs to a region of the female body characterized by a mucosal junction called a squamo-columnar junction (SCJ). There is no doubt that the area near this junction, the transformation zone (TZ) (Reich, Regauer, McCluggage, Bergeron, & Redman, 2017), is the critical location for cervical infections, metaplasia and carcinogenesis. The TZ is characterized physiologically by a so-called metaplastic epithelium, including precursor or stem cells that are thought to be targets for disorders (Chumduri et al., 2021). Discussions about the embryonic origin of these stem or precursor cells led to a number of recent clinical studies revealing that knowledge of some aspects of the development of the vagina and the cervix in humans remains incomplete. Martens, Arends, Van der Linden, De Boer, and Helmerhorst (2004), Martens et al. (2007, 2009) and Herfs et al. (2012, 2013) describe different kinds of adult reserve or precursor cells of (respectively) p63/CK17-positive or CK7-positive phenotypes with reference to their prenatal occurrence and distribution. As neither group refers strictly to the anatomically accepted terminology, we do not know where they found these prenatal stem cells; moreover, they did not reveal their origins. Recently, our group (Fritsch, Richter, & Adam, 2012; Fritsch, Hoermann, Bitsche, Pechriggl, & Reich, 2013) and that of Cunha and colleagues (Cunha et al., 2017; Cunha et al., 2018; Robboy, Kurita, Baskin, & Cunha, 2017) have conducted basic morphological research on the epithelial differentiation of the entire human utero-vaginal anlagen. A member of the latter group (Kurita, Cooke, & Cunha, 2001) clearly distinguished “organogenetic” differentiation of the cervical epithelium, defined as a process by which the Müllerian epithelium is differentiated, from “functional” differentiation, defined as a process during estrous cycles. He considered correct organogenetic differentiation to be a prerequisite for correct functional differentiation. This is in agreement with the German gynecologist Meyer (1910), who began his pioneering work on the epithelial development of the cervix and portio uteri with the statement that pure knowledge about development is rather interesting, but without this knowledge, discussion of pathology in the adult is not firmly grounded. In 1910, Meyer made a complete study of all the different prenatal stages. His thorough histological work revealed cells and groups of cells in the region of the TZ that could be the stem cells we speak of nowadays. Meyer, like Pixley (1976), denied the existence of a metaplastic epithelium during development. Apart from rare citations (Carmichael & Jeaffreson, 1949), Meyer’s important ideas have not been respected in the Anglo-American literature. The term “metaplastic epithelium” is fixed in the clinical literature. Thus, one major focus of the present study on fetal stages and newborns, as examined in previous papers, is to contribute to clarifying the prenatal existence, organogenetic development and composition of the TZ, and the distribution and origin of the reserve/precursor cells.

As shown by Chumduri et al. (2021), epithelial homeostasis in the area of the TZ is maintained by alternative signals from the underlying

stromal compartment driving the differential proliferation of the respective cell lineages. Therefore, a second focus of our study is to analyze the organogenetic development of the mesenchymal surroundings of the portiocervix and the vaginal fornices. Furthermore, insights into newborn specimens under the influence of maternal steroids will indicate the morphological changes during functional differentiation.

2 | MATERIALS AND METHODS

2.1 | Human fetal and newborn specimens

A total of 29 female embryos and fetuses between 20 and 34 weeks of development, and two female newborns used in former studies (Fritsch et al., 2012; Fritsch et al., 2013; Fritsch, Zehm, Illig, Moser, & Aigner, 2010), were examined. The specimens were obtained from the archival collection of the Division of Clinical and Functional Anatomy, Innsbruck Medical University. None of them showed macroscopic abnormalities. They were categorized according to their postovulatory/postfertilization age (O’Rahilly & Müller, 2010) based on crown-rump length (CRL) and external and internal morphology, or on their estimated gestational age.

2.2 | Tissue preparation and conventional histology

The specimens were immediately fixed by immersion in cold 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, for 24 hr followed by rinsing in PBS. After dehydration and embedding in paraffin wax (Paraplast Regular, Sigma-Aldrich, St. Louis, MO) using a routine histological infiltration processor (Miles Scientific Inc., Naperville, IL) the specimens were cut into 4 µm thick serial sagittal or transverse sections using a Microm ERGO Star Rotations microtome (Microm, Walldorf, Germany). The sections were mounted on glass slides (SuperFrost Plus; MenzelGläser, Braunschweig, Germany) and air-dried overnight followed by incubation for 2 hr at 60°C to adhere the sections firmly to the glass slides. Every 10th section of a series was dewaxed with xylene, rehydrated in a graded alcohol series and stained with hematoxylin and eosin for cytoarchitectural orientation.

2.3 | Antisera

The hosts, dilutions and sources of the primary antibodies used in this study and for heat-induced epitope retrieval (HIER) when required are listed in Table 1.

2.4 | Immunohistochemistry

Immunohistochemistry was performed on paraffin sections in a Ventana Roche Discovery XT Immunostainer (Mannheim, Germany)

TABLE 1 Antibodies used in immunohistochemistry

Antibody (catalog number)	Host	Dilution in IHC	HIER	Supplier	Marker for
CD31 (JC70) (760-4,378)	Mouse	Ready to use	CC1 standard	Cell Marque (Sigma-Aldrich) Rocklin, CA, USA	Non-neoplastic and neoplastic vascular endothelial cells; (Parums et al., 1990)
Cytokeratin 7 (SP52) (790-4,462)	Rabbit	Ready to use	CC1 standard	Ventana (Roche), Tucson, Arizona, USA	Most ductal, glandular and transitional epithelia; normal and neoplastic cells of ovary, lung and breast epithelial origin; (Roche & Hsi, 2002; Rubin, Skarin, Pisick, Rizk, & Salgia, 2001; Sack & Roberts, 1997)
Cytokeratin 14 (RTU-LL002)	Mouse	Ready to use	CC1 mild	Novocastra, Newcastle upon Tyne, UK	stratified epithelial cell types (Purkis, Steel, MacKenzie, Nathrath, & Leigh, 1990)
Cytokeratin 17 (790-4,560)	Rabbit	Ready to use	CC1 standard	Ventana (Roche), Tucson, Arizona, USA	Basal/stem cells in complex epithelia (Smedts et al., 1992b)
Laminin (AR078-5R)	Rabbit	Ready to use	Without CC; Protease 3 pretreatment	BioGenex, Fremont, Canada	Basement membranes; morphological change in basement membranes (invasive tumors);
p63 (MS-1084-P)	Mouse	1:400	CC1 standard	Termo Fisher Scientific, Waltham, Massachusetts, USA	Basal/progenitor cells of many epithelial tissues (Yang et al., 1999)
SMA (E046)	Mouse	Ready to use	Without pretreatment	Linaris, Dossenheim, Germany	Smooth muscle actins, myofibroblasts and myoepithelial cells (Skalli et al., 1986)
Vimentin (V9) (790-2,917)	Mouse	Ready to use	CC1	Ventana (Roche), Tucson, Arizona, USA	Endothelial cells, fibroblasts, smooth muscle cells and lymphoid cells. Some tumors co-expressing vimentin and cytokeratin like ovarian carcinomas, some renal carcinomas, thyroid carcinomas, etc.

according to the standard DAB-MAP discovery research procedure. If required, antigen retrieval was initiated by heat-induced unmasking of the epitopes while the slides were immersed in accordance with the manufacturer's instructions (short, mild or standard for different incubation times) in EDTA buffer (Cell Conditioning Solution CC1, Ventana 950-124). After incubation with primary antibodies for 1 hr at 37°C, the specimens were treated with a biotinylated immunoglobulin cocktail of goat anti-mouse IgG, goat anti-mouse IgM, goat anti-rabbit IgG and protein block (Discovery Universal Secondary Antibody, Ventana 760-4205) for 30 min at room temperature. The DAB-MAP Detection Kit (Ventana 760-124) was used for detection by the diaminobenzidine development method with copper enhancement followed by light counterstaining with hematoxylin (Ventana 760-2021) for 4 min. The sections were then manually dehydrated, cleared in xylene, and cover-slipped. The immunohistochemical staining reaction was referred to positive controls (skin, tonsil, bone marrow, prostate) in each experiment. For additional controls, representative sections were processed in the same way as previously described but omitting the primary antibodies or substituting isotype matching immunoglobulins for them. These controls were consistently negative.

We applied the following antibodies to characterize different epithelia: p63 for basal epithelial progenitor cells, cytokeratin 7 for

glandular and transitional epithelia, cytokeratin 14 (comparable to cytokeratin 5) for stratified squamous epithelia and cytokeratin 17 for basal progenitor cells in complex epithelia. We also used the following antibodies to characterize the mesenchymal surroundings: SMA to give an overview of smooth muscle cell differentiation, laminin to reveal the connection between the cells and the extracellular matrix, vimentin to show epithelio-mesenchymal transitions and CD 31 to identify the growth and spread of vessels. All those antibodies were well established for our paraformaldehyde-fixed fetal specimens (Fritsch et al., 2013).

The DAB-MAP Detection Kit was used to detect p63 immunostaining, and vimentin immunohistochemistry was recognized using the RedMap Detection Kit (Ventana 760-123), a streptavidin-biotin alkaline phosphatase detection system with fast red detection. Denaturation between single immunostainings was ensured by heat treatment at 85°C for 8 min.

2.5 | Image analysis for immunohistochemistry

Digital images of immunostained slices were acquired in AxioVision microscope software linked to an AxioCamHRc color camera and an AxioPlan 2 microscope (Zeiss, Jena, Germany). The immunohistochemical

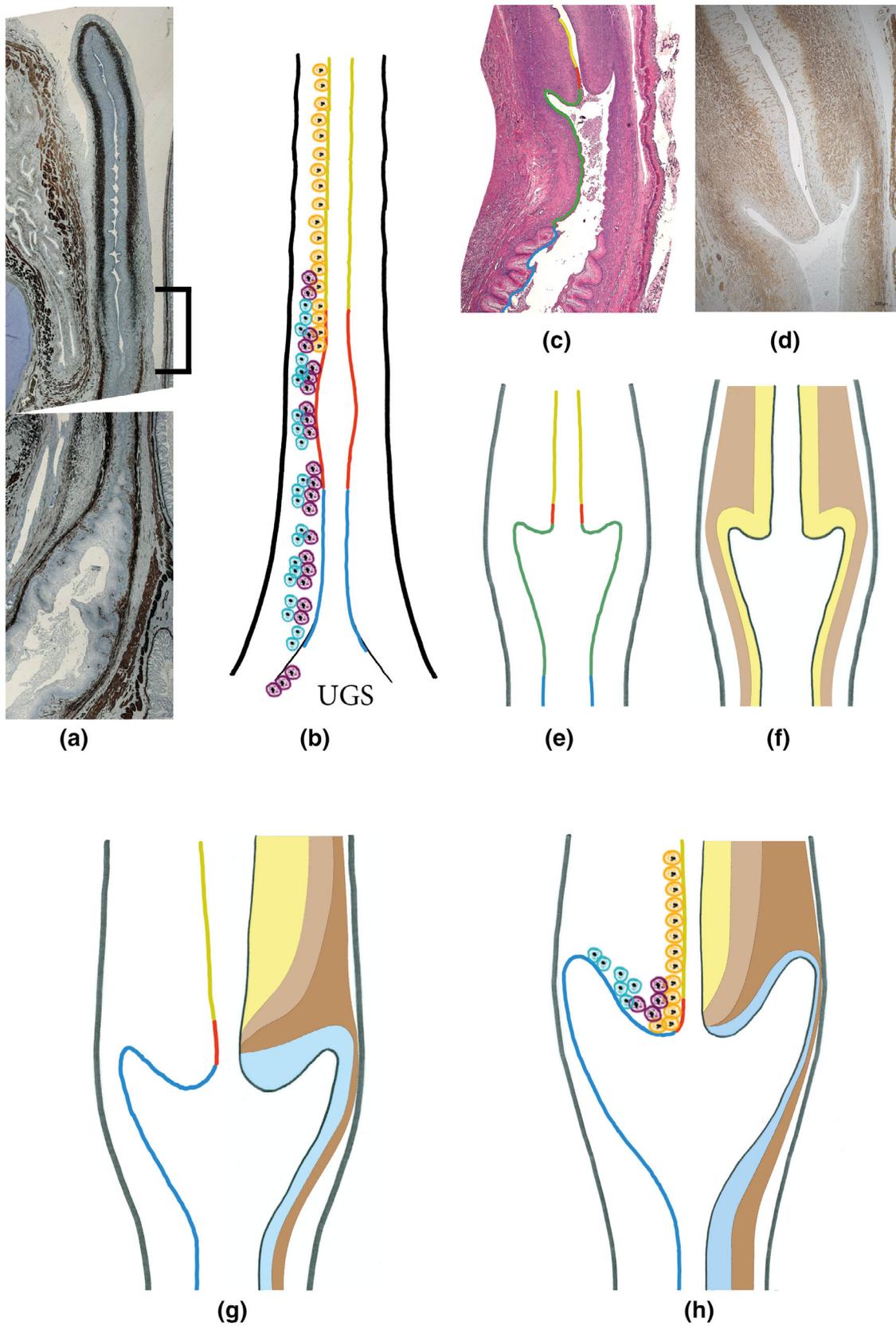


FIGURE 1 Legend on next page.

staining pattern was examined particularly for the muscular coat, epithelial borders and the underlying mesenchymal surroundings.

3 | RESULTS

As the focus of this study is on the epithelial and mesenchymal shaping of the portiocervicis and the vaginal fornices, we include preparations from prenatal week 20 up to the newborn stage, when the sinovaginal bulb and vaginal plate have already disappeared (Cunha et al., 2018; Sadler, 2019).

In 20-week-old fetuses, the regions of the future vaginal fornix and the future portiocervicis were indicated by different muscular coats of the uterus, cervix and vagina (Figure 1a). As recently reported (Fritsch et al., 2012, 2013), a lumen connected to the urogenital sinus (UGS) was found in the lower part of the vagina. The upper (still solid) part of the vagina and the future cervix uteri were still part of a common utero-vaginal tube derived from the Müllerian (paramesonephric) ducts and covered by differentiating Müllerian epithelium. The predominant cell type within the basal and supra-basal layers of the squamous epithelium of the lower vagina and the basal layers of the stratified uncategorized cuboidal epithelium of the upper vagina were p63- and CK14-positive cells (Figure 1b), which we assume are co-expressed. These cells stopped abruptly in the lower cervical canal (Figures 1b and 2d). Furthermore, p63- and CK17-positive cells, which we assume are co-expressed, were found basally as densely packed groups in the lower and as scattered groups in the upper vagina (Figure 1b). The p63- and CK17-positive cells originate/ascend from the endodermal derived UGS (Figures 1b and 2a,b). Scattered cells of this type were also observed in the lower cervical canal (Figure 2c,e). CK7-positive columnar cells (comparable to the CK8 of previous studies) were the predominant cell type in the cervical epithelium (Figure 1b). Serial sections revealed no co-expression of CK7 with p63. CK7 positivity was also found in the flat apical cell layers of the upper vagina (Figure 2f). We did not recognize differences in the still-unstructured stroma underlying the incomplete differentiated epithelia.

In 25-week-old fetuses, the vaginal fornix and the portiovaginalis cervicis were discernible owing to their developing characteristic forms (Figure 1d,e). CK14-, CK17- and p63-positive cells were found in all vaginal epithelia, that is, the squamous epithelium of

the lower vagina and the stratified cuboidal epithelium of the upper vagina, which was temporarily vascularized and perforated (Figure 2g,h). CK7 positivity was restricted to the endocervical epithelium (Figure 1e).

There were three borders between the different epithelia (Figure 1e): one between the squamous epithelium of the lower and the stratified, vascularized cuboidal epithelium (blue/green) of the upper vagina; one between the latter and a small area of a low stratified epithelium at the caudal end of the cervical canal (green/red); and a third at the caudal end of the CK7-positive cervical epithelium (red/yellow). Two stromal zones could be detected adjacent to the epithelia of the cervix and the vaginal fornix: Zone 1 (Figure 1f: yellow) directly connected to the epithelium, with a high density of vimentin-positive mesenchymal cells; and Zone 2 (Figure 1f: light brown) characterized by a high density of smooth muscle cells. Both zones extended from the cervical canal to the border between the upper and lower vagina.

Within older (third trimester) fetuses, adult type epithelia were identified in the vaginal fornix and the portiocervicis. The temporarily vascularized epithelium of the upper vagina had disappeared and been replaced; thus, the whole vagina including the ectocervix was covered with squamous epithelium (Figure 1g left), the cells of which still expressed CK14 but had lost their CK17 expression (Figure 2i,j). However, the latter increased in the cervical low stratified epithelium adjacent to the squamous epithelium (Figure 2j). CK7 positivity was still predominant in the cervical canal (Figure 2k), whereas flat luminal CK7-positive cells were rarely found in the upper vagina (Figure 2k). Two epithelial borders were detectable, both situated within the cervical canal (Figure 1g left): a caudal one between the squamous and the low stratified epithelium and a cephalad one between the latter and the columnar cervical epithelium. Differentiation of the stroma had advanced: Zone 1 with high cell density was restricted and adjacent to the CK7-positive cervical epithelium (Figure 1g right, Figure 3a). Zone 2 (Figure 1g right) could be divided into two sub-zones: zone 2.1 (Figure 3b) characterized by densely packed smooth muscle cells, and zone 2.2 (Figure 3c) by more loosely packed smooth muscle cells and a high density of blood vessels. Zone 2 was situated underneath the low stratified epithelium. Zone 3 (Figure 1g right, Figure 3d) was characterized by a high amount of extracellular matrix and lower cell density and was adjacent to the squamous epithelium.

FIGURE 1 Overviews and schematic drawings of the utero-vaginal anlagen with special emphasis on the portiocervicis and the vaginal fornix. (a) Sagittal section through the utero-vaginal anlagen of a 20-week old female fetus. SMA labeling. Scale bar 100 μ m. Bracket: region of the future portiocervicis. (b) Schematic drawing indicating the epithelial borders of UGS, vagina and cervix and the distribution of the important cell types in a fetus about 20 weeks old. (c) Sagittal section through the utero-vaginal anlagen of a 25-week-old female fetus. HE staining. Scale bar 100 μ m. (d) Sagittal section through the utero-vaginal anlagen of a 25-week-old female fetus. Laminin labeling. Scale bar 500 μ m. (e) Schematic drawing of the utero-vaginal anlagen of a 25-week-old female fetus indicating the epithelial situation. (f) Schematic drawing of the utero-vaginal anlagen of a 25-week-old female fetus indicating the stromal situation. (g) Schematic drawing of the utero-vaginal anlagen of a 30-week-old female fetus indicating the epithelial situation on the left side and the stromal situation on the right. (h) Schematic drawing of the utero-vaginal anlagen of a newborn indicating the epithelial situation on the left side and the stromal situation on the right. Cells: blue cells = CK 14; violet cells = p63/CK 17 co-expressed cells; yellow cells = CK 7. Lines: blue = vaginal squamous epithelium; red = stratified uncategorized epithelium (TZ in older stages) green = stratified uncategorized but vascularized epithelium; yellow = cervical columnar epithelium. Areas: yellow = zone 1; light brown = zone 2; zone 2.1; brown = zone 2.2; blue = zone 3

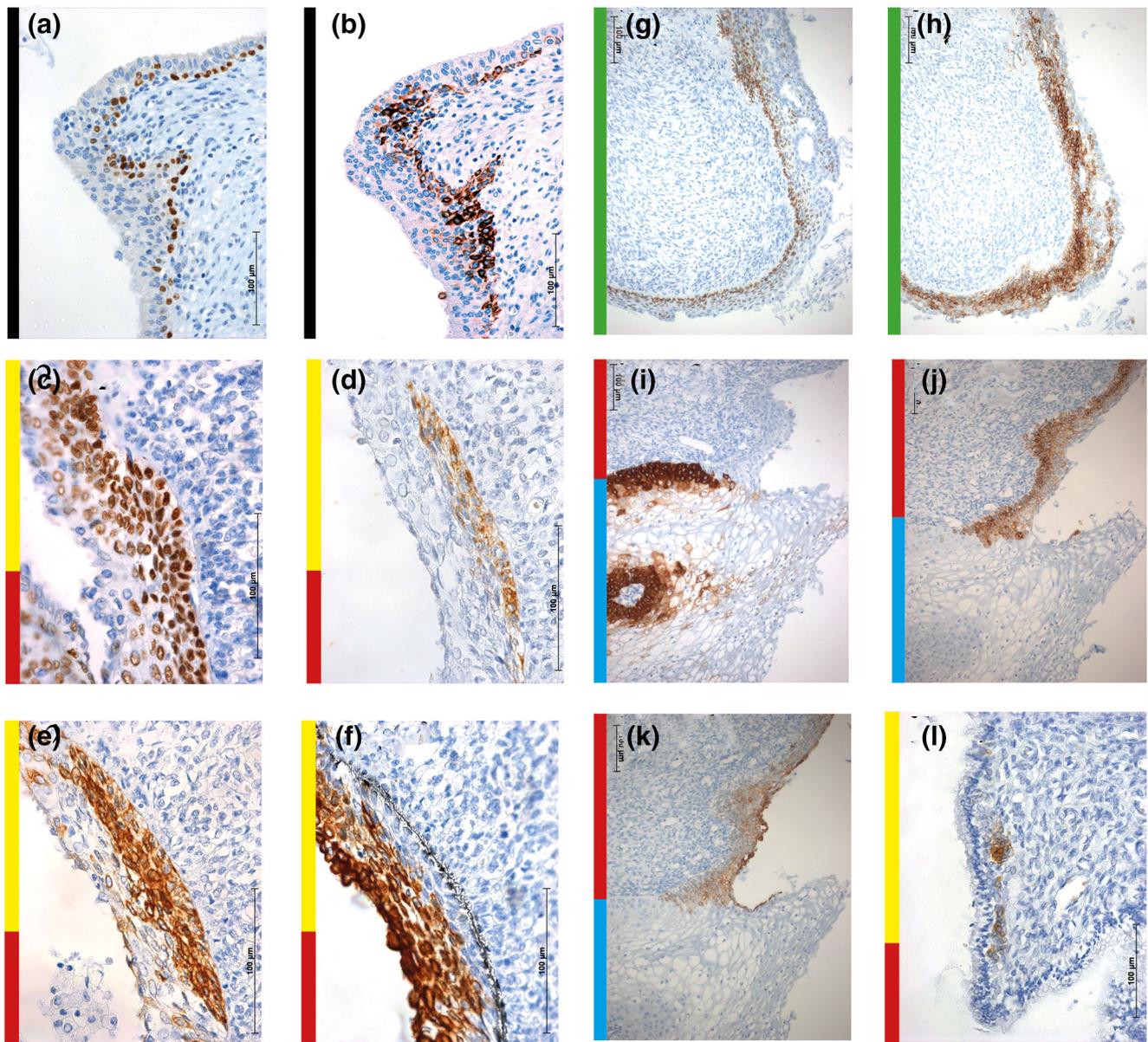


FIGURE 2 Histological details of the epithelia of the utero-vaginal anlagen with special emphasis on the portiocervicis and the vaginal fornix. (a,b) Enlargements of sagittal sections of the urogenital sinus in a 20-week-old fetus. (a) p63; (b) CK 17. Scale bar 100 µm. (c–f) Enlargements of sagittal sections of the future border of the cervical columnar epithelium in a 20-week-old fetus. (c) p63; (d) CK 14; (e) CK 17; (f) CK 7 basement membrane dotted by a black line. Scale bar 100 µm. (g,h) Enlargements of sagittal sections of the vaginal fornix and the temporarily vascularized stratified cuboidal epithelium in a 25-week-old fetus. (g) p63; (h) CK 17, scale bar 100 µm. (i–k) Enlargements of sagittal sections of the SCJ in a 29-week-old fetus. (i) CK 14, (j) CK 17, (k) CK 7. Scale bar 100 µm. (l) Enlargement of a sagittal section of the lower cervical epithelium in a newborn. A group of CK 17 cells is marked. Scale bar 100 µm. Lines: black UGS; blue = vaginal squamous epithelium; red = stratified uncategorized epithelium (TZ in older stages) green = stratified uncategorized but vascularized epithelium; yellow = cervical columnar epithelium

In the newborn, the fornix and the portiocervicis were completely established. Small groups of CK17-positive cells were restricted to the low stratified epithelium of the cervix (Figure 1h left, Figure 2l) and the neighboring CK7-positive cervical epithelium. In perinatal stages, the position of the border (blue/red) between the squamous and the low stratified epithelium had descended towards the external os of the cervix.

The stromal zones were the same as described for the older fetal stages (Figure 1h right, Figure 3e–h). Their positions had changed according to the epithelial shift towards the external os.

4 | DISCUSSION

The present study was undertaken to focus on the development and differentiation of the portiocervicis and the vaginal fornix. We report that in mid-term fetuses the shape of the portiocervicis and the vaginal fornix can first be differentiated because of the different muscular coats and of the uncategorized Müllerian-derived epithelium, which is rapidly reconstructed into a multilayered squamous epithelium. Only thereafter does organogenetic differentiation of the epithelia and the

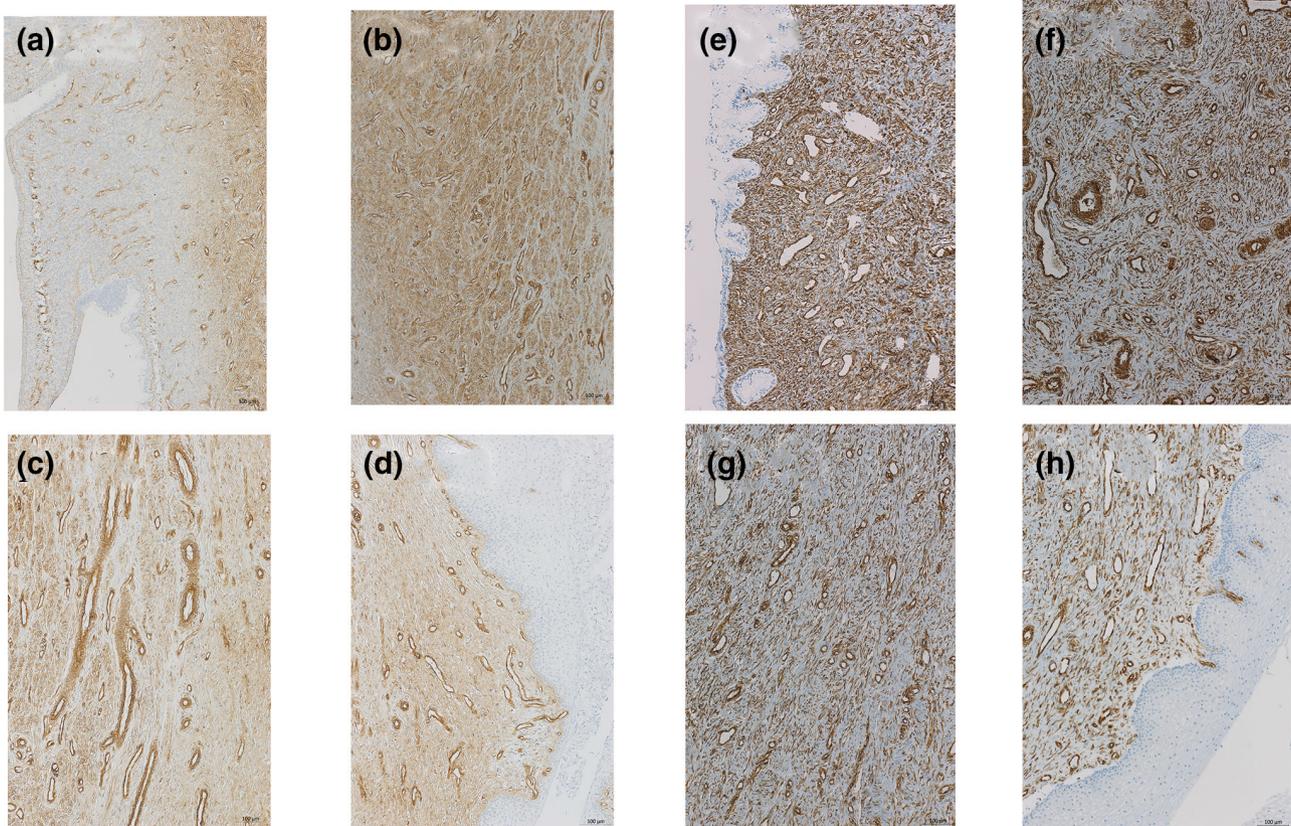


FIGURE 3 Histological details of the stroma of the utero-vaginal anlagen with special emphasis on the portiocervix and the vaginal fornix. (a–d) Enlargements of sagittal sections of the portiocervix in a 29-week-old fetus, laminin labeling. (a) Zone 1, (b) Zone 2.1, (c) Zone 2.2, (d) Zone 3: Scale bars 100 µm. (e,f) Enlargements of sagittal sections of the portiocervix in a newborn, vimentin labeling. (e) Zone 1, (f) Zone 2.1, (g) Zone 2.2, (h) Zone 3: Scale bars 100 µm

underlying stroma of the cervical canal take place in older fetuses. As a result of this process, initial functional differentiation can be observed during perinatal stages.

However, it is necessary to consider the results described here as one piece in the puzzle of the development of the entire utero-vaginal anlagen. If we want to understand the origins of the precursor cells and where they are definitely situated, and whether a TZ and a so-called metaplastic epithelium exist prenatally, and how epithelial and stromal borders change, we must look at the complete spatiotemporal development of the utero-vaginal anlagen and include the results of our previous papers (Fritsch et al., 2012; Fritsch et al., 2013).

4.1 | Precursor cells

We learned that p63-positive cells, which are considered to be reserve cells in the vaginal and cervical epithelium (Ince et al., 2002; Kurita & Cunha, 2001), ascend from the UGS towards the lower vagina during early stages (Fritsch et al., 2012), and later towards the upper vagina, the fornix and the portiocervix (Fritsch et al., 2013). As we pointed out in the present study, these cells are supposed to co-express CK14 and/or CK17 in mid-term fetuses. Thus, we are the first to show that so-called reserve or precursor cells with an overlap of

p63 and CK17 (Martens et al., 2007) are derived from the UGS, not from the Müllerian epithelium as supposed by Martens et al. (2007). Furthermore, we can demonstrate the spatio-temporal distribution of these cells during fetal life: in early stages, they are situated basally in the vagina, then they ascend towards the cervix; and during late fetal life and in the newborn they are restricted to the region of the TZ and the directly neighboring cervical epithelium. Martens et al. (2009) believed that these reserve cells are capable of dual differentiation: on the one hand to a squamous-type epithelium and on the other to a columnar-type epithelium. However, we believe that p63/CK17 cells are only able to differentiate to a squamous-type epithelium.

Moreover, we are the first to show clearly that CK7-positive cells, which were considered to be cervical precursor or stem cells by Herfs et al. (2012) and Herfs, Soong, Delvenne, and Crum (2017), are of Müllerian origin. These are prominent cells within the cervix; furthermore, they are situated in the TZ and luminally at the SCJ. Sometimes we found such luminal cells even in the well-differentiated vagina (not shown). Our findings are consistent with the commonly accepted opinion that CK7 staining is diffuse and uniform in the adult cervical epithelium (Moll, Franke, Schiller, Geiger, & Krepler, 1982; Smedts et al., 1992a; Smedts et al., 1992b). We do not agree with the proposal by Herfs et al. (2012) that these cells are the discrete population responsible for squamous metaplasia. We suppose they could be the

origin of adenocarcinomas, and they could be one of the two stem cell population types in the TZ. As we describe both precursor or stem cell types, the CK17 stem cells according to Martens et al. (2004, 2007) and the CK7 cells according to Herfs et al. (2012) within the TZ, along with two stromal zones meeting here, our descriptive results of human fetal development strongly support the new hypothesis of Chumduri et al. (2021) that two different cell lineages meet at the TZ, and that in the event of HPV infection they depend on alternative signals from the stromal compartment rather than there being a transition from one epithelial type to another.

4.2 | Transformation zone and so-called metaplastic epithelium

During the female reproductive years the vaginal fornices and most of the ectocervix are covered with well-differentiated squamous epithelium. In most cases, this is followed by an area of so-called transformed metaplastic epithelium, called the TZ, which is followed cranially by the cervical columnar epithelium. Colpologists describe the borders between the epithelia as the original and new SCJ (Reich et al., 2017). We consider the TZ to be a small region of uncategorized (according to histological categories) epithelium during fetal life, resembling the Müllerian epithelium in “reconstruction,” and capable of differentiating into either squamous or columnar epithelium owing to its different precursor cells (see above). Thus, the TZ described here is a physiological transformation zone between the squamous and columnar epithelia (Singer, Khan, & Borstein, 2014). It is accompanied by a border between two different stromal zones. In accordance with the literature (Pixley, 1976; Singer et al., 2014), the best term for this epithelium is “original metaplastic epithelium.” Like the other authors, we are aware that according to a strict interpretation of “metaplasia,” the epithelium is not metaplastic.

4.3 | Epithelial and stromal borders

In morphology textbooks (Standring, 2008 in Gray's Anatomy) the SCJ is defined as the point where the secretory epithelium of the cervical canal meets the stratified squamous epithelium. We are the first to point out that the definitive cephalad border of the SCJ (red/yellow) is already fixed during early fetal stages. It is marked by an overlap between p63 and CK14 positivity and is situated in the cervical canal (Figure 1b). We interpret this as follows: the endocervical cephalad border for squamous epithelium is thereby fixed for life, that is, differentiated squamous epithelium will never be found upwards from this border in the healthy cervix.

In midterm fetuses, the ascending vaginal squamous epithelium borders undifferentiated Müllerian epithelium (blue/green). The border between these epithelia is situated within the vagina (Figure 1e left). Owing to the ascending differentiation, this border vanishes during late fetal life (blue/red). Thus, it is important to recognize that the border of the ascending squamous epithelium is not identical with the

definite SCJ. However, we believe that other authors (Herfs et al., 2012, 2013; Martens et al., 2007) did not take this difference into account when they studied the positions of precursor cells. As already described by Meyer (1910), the SCJ and the TZ descend towards the external os of the cervical canal in the newborn (Figure 1h left). They are accompanied by a stromal interaction characterized by a broadening of stromal zone 2 (Figure 1h right). This process can be considered the first functional shift (maternal hormones?). After birth, the position of the SCJ reverts into the cervical canal until puberty (Pixley, 1976) when estrogen levels rise.

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