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New insights into human female reproductive tract development

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ABSTRACT

We present a detailed review of the embryonic and fetal development of the human female reproductive tract utilizing specimens from the 5th through the 22nd gestational week. Hematoxylin and eosin (H & E) as well as immunohistochemical stains were used to study the development of the human uterine tube, endometrium, myometrium, uterine cervix and vagina. Our study revisits and updates the classical reports of Koff (1933) and Bulmer (1957) and presents new data on development of human vaginal epithelium. Koff proposed that the upper 4/5ths of the vagina is derived from Müllerian epithelium and the lower 1/5th derived from urogenital sinus epithelium, while Bulmer proposed that vaginal epithelium derives solely from urogenital sinus epithelium. These conclusions were based entirely upon H & E stained sections. A central player in human vaginal epithelial development is the solid vaginal plate, which arises from the uterovaginal canal (fused Müllerian ducts) cranially and squamous epithelium of urogenital sinus caudally. Since Müllerian and urogenital sinus epithelium cannot be unequivocally identified in H & E stained sections, we used immunostaining for PAX2 (reactive with Müllerian epithelium) and FOXA1 (reactive with urogenital sinus epithelium). By this technique, the PAX2/FOXA1 boundary was located at the extreme caudal aspect of the vaginal plate at 12 weeks. During the ensuing weeks, the PAX2/FOXA1 boundary progressively extended cranially such that by 21 weeks the entire vaginal epithelium was FOXA1-reactive and PAX2-negative. This observation supports Bulmer's proposal that human vaginal epithelium derives solely from urogenital sinus epithelium. Clearly, the development of the human vagina is far more complex than previously envisioned and appears to be distinctly different in many respects from mouse vaginal development.

1. Introduction

The development of the human female reproductive tract and especially knowledge concerning the germ cell layer derivation of vaginal epithelium rests on histological, histochemical observations and to limited, immunohistochemical investigations. The seminal paper on this topic by Koff appeared in 1933 when the technical state of the art was tissue sections 25–100 μ thick stained with hematoxylin and eosin (Koff, 1933). From this humble beginning, the field has progressed to elegant epithelial cell lineage tracing to reveal the relative contribution of epithelium of the urogenital sinus (UGS) versus the Müllerian (paramesonephric) ducts to vaginal epithelial development in mice (Kurita, 2010) and immunohistochemical studies in man (Fritsch et al., 2013, 2012). The current paper and its two companions (a) review the collective trove on human female reproductive tract development (Bulmer, 1957; Cai, 2009; Forsberg, 1996, 1973; Fritsch et al., 2013, 2012; Hunter, 1930; Koff, 1933; Konishi et al., 1984; Kurita, 2010; Mutter and Robboy, 2014; O'Rahilly, 1977, 1983; O'Rahilly and Muller, 1992; Reich and Fritsch, 2014; Sinisi et al., 2003; Sulak et al., 2007), (b) provide new data on the derivation of human vaginal epithelium and (c) highlight the role of differentiation markers and signaling pathways involved in human uterovaginal development.

Both the male and female reproductive tracts collectively consist of the gonads, their internal ductal systems, and the external genitalia. The embryonic anlagen for these elements appear during the first trimester, followed by extensive growth and maturation during the fetal period and postnatally. Ovarian development occurs in fetuses with the XX chromosome pair, while testicular development occurs in fetuses with XY chromosomes. This review focuses on the developing female internal genitalia (uterine tube, uterine body, cervix, and vagina). It omits discussion of the molecular genetic basis of both normal (Mutter

Abbreviations: H & E, hematoxylin & eosin; UGS, urogenital sinus; MDE, Müllerian duct epithelium; UGE, urogenital sinus epithelium

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Table 1
Normal development of the Müllerian and its derivatives [a].

Week of gestationpost ovulation	Crown-rump (mm)	Heel-toe length (mm)	Carnegie Stage	Developmental event
3 wk	2.5 mm			Pronephric tubules form; pronephric (mesonephric) duct arises and grows caudad.
4 wk	3–5 mm		Stage 12	Pronephros degenerated, but mesonephric duct reaches cloaca.
5 wk	7 mm			
6 wk	11 mm		Stage 17	Müllerian ducts appear as funnel-shaped opening of celomic epithelium.
7 wk	18 mm		Stage 22	Müllerian ducts migrate to about half distance to urogenital sinus.
	23 mm		Stage 23	Müllerian ducts extend caudally to near the urogenital sinus.
8 wk	30 mm		30 mm	Müllerian ducts begin midline fusion and make contact with urogenital sinus at the Müllerian tubercle.
	36 mm			
9 wk	50 mm		56 mm	Müllerian ducts fuses (entire septum gone); Epithelium lining uterovaginal canal stratifies (1–2 cells layers thick).
10 wk	60 mm	2–5 mm	60 mm	
11 wk	71 mm	5–8 mm	63 mm	Bilateral sinovaginal bulbs (evagination of UGS) appear.
	75 mm		75 mm	Vaginal plate first seen distinctly at 75 mm (complete at 140 mm; week 17).
12 w	93 mm	8–11 mm		
13 wk	105 mm	12–14 mm	100 mm	
14 wk	110–140 mm116 mm	15–17 mm	110–140 mm	Marked growth of caudal vagina (between 110 & 140 mm).
			130 mm	Vaginal rudiment reaches level of vestibular glands; uterovaginal canal (15 mm total length) divisible into vagina (one-half), cervix (one-third), and corpus (one-sixth); boundaries ill defined. Endometrial stromal & myometrial layers of uterus become apparent. Solid epithelial anlage of anterior and posterior fornices appear Vagina begins to show slight estrogen (epithelial squamous differentiation)
15 wk	130 –140 mm	18–21 mm	135 mm	Cervix about 5 mm long
			139 mm	Uterine (Fallopian) tube begins active growth phase
			140 mm	Vaginal plate extends from vestibule to endocervical canal.
16 wk	142 mm	21–24 mm		Uterine/cervical glands begin as outpouchings of simple columnar epithelium
			151 mm	Vaginal plate longest and begins to canalize.
			151 mm	Solid epithelial projections of anterior and posterior fornices demarcate cranial end of vagina.
17 wk	153–162 mm	24–27 mm	160 mm	Palmate folds of cervix appear (forerunner adult cervix).
			162 mm	Mucoid development of cervix begins. Estrogen-induced thickening of vaginal epithelium.
18 wk	164 mm	27–30 mm	170 mm	Fornices hollow.
19 wk	177 mm	31–33 mm	180 mm	Cavitation of vaginal canal completed.
			185 mm	Uterine tube growth marked (~3 mm/week to week 34).
			190 mm	Cervix about 10 mm long.
21 wk	197 mm	37–40 mm		
22 wk	208 mm	40–43 mm	200 mm	Vagina completely formed.
			210 mm	Differentiation of muscular layer of uterus complete.
			227 mm	
24 wk	215–295	47–49 mm		Uterine fundus well defined; uterus assumes adult form. Uterine body about 10 mm long.
25 wk	227			
26 wk	240			Cervix about 20 mm long.
34 wk	305			Cervix about 25 mm long.
38 wk	362 mm			Birth.

NOTE: Week 1 includes = days 1–7, week 2 = days 7–14, etc.
[a] Table compiled from Koff (1933), Mutter and Robboy (2014), O'Rahilly (1977, 1983), O'Rahilly and Muller (1992, 2010).

Table 2
Definition of terms referred to in human and mouse vaginal development.

Human terms	Definition
Uterovaginal canal (Koff, 1933)	The fused Müllerian ducts located in the pelvic midline.
Müllerian tubercle (Fritsch et al., 2013; Koff, 1933)	The Müllerian tubercle is the initial point of contact of the Müllerian ducts and with the UGS.
Sinovaginal bulb (Koff, 1933; Shapiro et al., 2000)	Cranial expansion of urogenital sinus epithelium that contributes to the vaginal plate
Vaginal plate (Koff, 1933)	A solid epithelial plate in the caudal uterovaginal canal
Mouse terms	
Müllerian vagina (Kurita, 2011)	The cranial portion of the perinatal mouse vagina. The epithelium, which transforms from simple columnar to stratified squamous, is derived from the fused Müllerian ducts
Sinus vagina (Kurita, 2011)	A solid dorsal process of the urogenital sinus that joins with the Müllerian vagina.

and Robboy, 2014) and abnormal gonadal sexual determination and the developing gonads themselves (Robboy and Mutter, 2014). Accordingly, this article summarizes knowledge of how female internal genitalia develop from their initial appearance in about week five through parturition (Table 1). The first of two companion articles amplifies the immunohistochemical changes that occur during human