

Age Related Morphological Changes in the Cervix of the West African Dwarf goat (*Capra hircus*)

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ABSTRACT

The cervix which changes with the reproductive cycle, history and age of the females, provides a natural communication between the uterus and the outside environment. This study investigated the chronological and morphological changes of the cervix of the West African Dwarf (WAD) goat during postnatal development. Forty-five female WAD goats ranging from birth, Weeks 2, 4, 8, 12, 16, 20, 24 and 28 of five goats each were used. The cervixes were exteriorised, examined and morphometric parameters obtained and then prepared for histological examinations. The cervix is a firm body in all the age groups and internally bore thick muscular cervical rings which increased with age. The straight cervical canal is straight and can be easily penetrated by an artificial insemination pipette. The gross morphometry of the cervix increased significantly ($p < 0.05$) with age. The lamina propria-submucosa lacked cervical glands. The tunica muscularis showed histological evidence of postnatal development with increase in age of WAD goat. The epithelium of the cervix was initially pseudostratified columnar at birth but became simple columnar by week 12. Periodic Acid Schiff (PAS) stain showed some mucus secretory activities at week 4. In conclusion, the study revealed that the cervix of WAD goat is not fully developed at birth but undergoes a postnatal development. The onset of secretory activities is associated with adenogenesis of uterine glands. Thus, the WAD goat probably attains puberty at week 16, earlier than previously reported.

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Introduction

The cervix of small ruminants has been described as an anatomically complicated sphincter-like structure at the base of the uterus consisting of a fibromuscular canal with multiple folds of tissues or rings (Colagross-Schouten *et al.*, 2014). Between species and breeds, there are great variations in the gross morphology of the cervixes. The cervical canal may either be straight or tortuous and contains mucosal annular rings which changes with the reproductive cycle, history and age (Kershaw *et al.*, 2005; Kaabi *et al.*, 2006; Dayan *et al.*, 2010; Robinson *et al.*, 2011). Through the cervix, the spermatozoa move into the uterine cavity and newborn moves into the vagina. Pathogens that affect the uterine cavity and fetuses also move through the cervix. The cervix is the first great barrier that functions as a sperm reservoir and serves for sperm selection (Heydon and Adams, 1979; Barros *et al.*, 1984; Katz *et al.*, 1997; Kölle *et al.*, 2010; Robinson *et al.*, 2011).

Assisted reproduction technologies are currently in use to improve genetics and reproductive output in small ruminants

as reproduction is an energy expensive process (Cognie *et al.*, 2003; Hansen, 2014). These are only possible in sheep and goats whose cervical morphology is well known as complications including damage to the cervix and puncture of the epithelium have been recorded (Campbell *et al.*, 1996). Studies have shown that the cervix of sheep is more difficult to penetrate by insemination pipette due to the number and irregular alignment of the cervical rings (Halbert *et al.*, 1990; Souza, 1993; Naqvi *et al.*, 2005; Kaabi *et al.*, 2006; Gultiken *et al.*, 2009; Cruz Júnior *et al.*, 2014). The tortuous nature of the ovine cervix had been a very major limiting factor to the development of artificial insemination and embryo transfer in the ovine species (Salamon and Maxwell, 1995; Rodríguez-Piñón *et al.*, 2018). More also, clinical problems have resulted when the cervix fails to dilate sufficiently or fails to dilate at all during parturition as in sheep with ring-womb (Dobson, 1988). The cervical morphology of the adult goat has been studied (Dayan *et al.*, 2010; Gupta *et al.*, 2011; Conde Junior *et al.*, 2012; Salih and Abass, 2014). However, little is known of the changes the goat cervix undergoes during postnatal growth. This study aimed to highlight the morphological changes of the cervix of the West African Dwarf (WAD) goat during postnatal development.

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Materials and methods

All animal procedures were carried out with approval from the University of Nigeria Nsukka Senate Committee on Medical and Scientific Research Ethics. Forty-five female WAD goats of known ages were used in this study. The female goats were sourced from local WAD goat breeders in Nsukka Local Government Area of Enugu state, Nigeria. The goats were purposively assigned to nine groups of five goats each including day old, weeks 2, 4, 8, 12, 16, 20, 24, and 28 of age. Each goat was weighed with a sensitive weighing balance (Model BR9010; Guangdong, China) and euthanized by intravenous injection of 70 mg/kg sodium pentobarbitone (Kyron Laboratories Ltd., Johannesburg, South Africa) at body weight. The cervixes were dissected out and trimmed of extraneous tissues. The length and weight of each cervix was determined. Cranio-caudal longitudinal incisions were made on the cervixes and the internal features examined and noted.

Histological preparation

Segments of the cervixes from each group were cut and fixed by immersion in Bouin's fluid for 24 hours. The segments were dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. 5 µm thick sections were obtained using a rotary microtome (Model 1512; Leitz®, Wetzlar, Germany) and were mounted on clean glass slides. The sections were stained with hematoxylin and eosin for general histological study as described by Suvarna *et al.* (2013). Sections were also stained with Periodic Acid Schiff (PAS) using Fast Green (FCF) as the counter-stain (Ikpegbu *et al.*, 2011) for identifying the presence of mucin in the cells. The sections were studied under the light microscope

and images captured using Moticam Camera 1000 (Motic China group Ltd., Xiemen, China).

Statistical analysis

The data generated were analyzed by one-way Analysis of Variance (ANOVA) using SPSS version 15 for windows and the results presented for each group as mean ± SEM. The variant means were separated by Duncan's multiple range test and significant differences were accepted at probability level of $P < 0.05$.

Results

In all the groups studied, the cervix appeared as a rigid thickened body between the body of the uterus cranially and the vagina caudally (Fig. 1). Within the cavity of the cervixes, the cervical rings were divided into cranial transverse rings separated by deep grooves and caudal funnel shaped circular rings, which protruded into the vagina as the portio vaginalis of the cervix. At birth, these rings were few and small (Fig. 1a). However, the cervical rings became more prominent and increased with age (Fig. 1b-1c) appearing as interdigitating regular arrangement of rings ranging from 5–7 in number. On these rings were numerous smaller longitudinal folds, which were indistinct at birth but became more prominent with age. The length and weight of the cervix of the WAD goat increased significantly ($p < 0.05$) from 0.64 ± 0.09 cm and 0.10 ± 0.01 g at birth to 2.44 ± 0.07 cm and 1.49 ± 0.11 g at week 28 respectively (Table 1). However, the relative weight of the cervixes to the overall weight of the animal did not increase significantly with age ($p > 0.05$).

Table 1. Age related changes in the mean length and weight of the cervix of WAD goat.

	Birth	Wk 2	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 24	Wk 28
Number of goats	5	5	5	5	5	5	5	5	5
Length (cm)	0.64 ± 0.09^a	0.75 ± 0.07^{ab}	0.96 ± 0.07^{abc}	1.06 ± 0.04^{bc}	1.10 ± 0.10^c	1.29 ± 0.18^{cd}	1.58 ± 0.10^d	2.00 ± 0.17^e	2.44 ± 0.07^f
Weight (g)	0.1 ± 0.0^a	0.12 ± 0.0^a	0.21 ± 0.02^a	0.43 ± 0.04^b	0.57 ± 0.06^b	0.83 ± 0.12^c	0.91 ± 0.06^{cd}	1.11 ± 0.08^d	1.49 ± 0.11^f
Relative organ weight	0.01 ± 0.00^{ab}	0.007 ± 0.00^a	0.01 ± 0.00^{ab}	0.016 ± 0.00^b	0.014 ± 0.00^b	0.016 ± 0.00^b	0.013 ± 0.00^{ab}	0.013 ± 0.00^{ab}	0.015 ± 0.00^b

Different superscripts abcdef in a row indicate significant difference ($P \leq 0.05$). Wk: Week

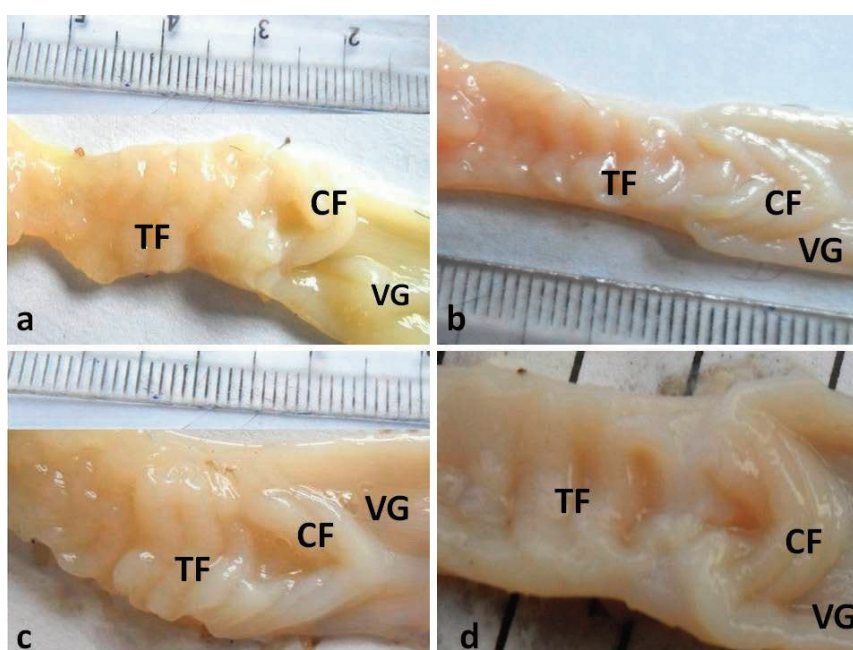


Fig. 1. Gross appearance of the mucosa of the cervixes of WAD goat at birth (a), week 12 (b), week 16 (c) and week 24 (d) showing the cranial transverse folds (TF) and the caudal circular folds (CF) of the cervix and the cranial vagina (VG). Note the numbers and sizes of the folds.

Histological features

The histology results showed that the cervix of a female WAD goat of all ages presented longitudinal cervical mucosal folds from which secondary and sometimes tertiary cervical folds extended from into the lumen (Fig. 2). The lamina propria of the tunica mucosa and the tunica submucosa blended to constitute the lamina propria-submucosa that extended and formed the cores of the cervical mucosal folds. At birth, the tunica muscularis was ill-defined (Fig. 2a) with less developed inner circular and outer longitudinal smooth muscles interspersed within a predominant connective tissue matrix. With increase in age, the tunica muscularis became more defined (Fig. 2b). At week 12, the inner circular and outer longitudinal

smooth muscle layers of the tunica muscularis were well developed and defined with less interspersed dense connective tissue (Fig. 2c). Blood vessels were observed within the dense connective tissue (Fig. 2d). From birth (Fig. 3a) to week 8 (Fig. 3b), the cervixes were lined by pseudostratified columnar epithelium. However, from week 12, the WAD goat cervix was at a well-advanced stage of development and the epithelium has changed to simple columnar epithelium with some of the columnar cells showing evidence of secretory activities (Fig. 3c). The secretory activities in the columnar cells increased with the age of the WAD goats (Fig. 3d). Cervical glands were not observed in the cervixes of the WAD goat in all the groups. Positive histochemical reactions with PAS stain were first observed at week 4 with few cervical epithelial cells at the base

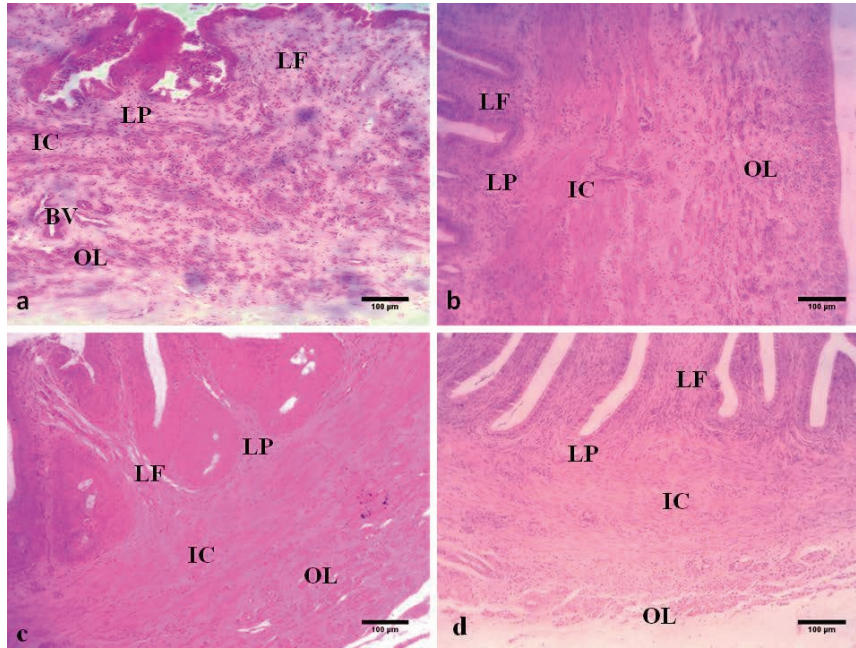


Fig. 2. Photomicrograph of the cervixes of WAD goat at birth (a), week 4 (b), week 12 (c) and week 24 showing the longitudinal cervical mucosal fold (LF), lamina propria-submucosa (LP) blood vessels (BV), inner circular (IC) and outer longitudinal (OL) smooth muscles. Note the progressive development of the tunica muscularis (IC and OL). H&E.

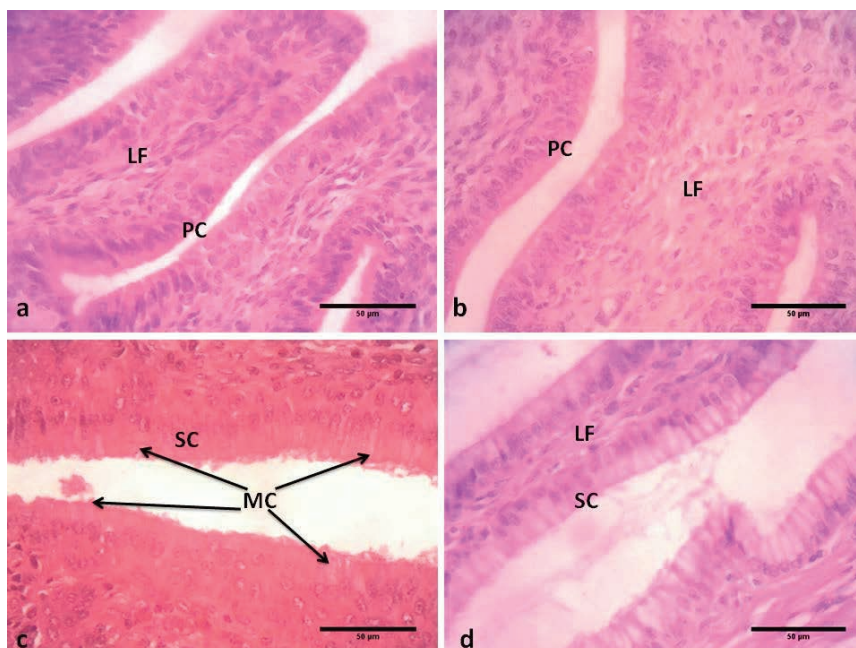


Fig. 3. Higher magnification of the longitudinal cervical mucosal folds (LF) of the cervixes of WAD goat at birth (a), week 8 (b), week 12 (c) and week 24 (d) showing the pseudostratified columnar epithelium (PC) during the early stages and the simple columnar epithelium during the later stages of development. Note that few epithelial cells (MC) at week 12 and all the epithelial cells (SC) at week 24 were secretory. H&E

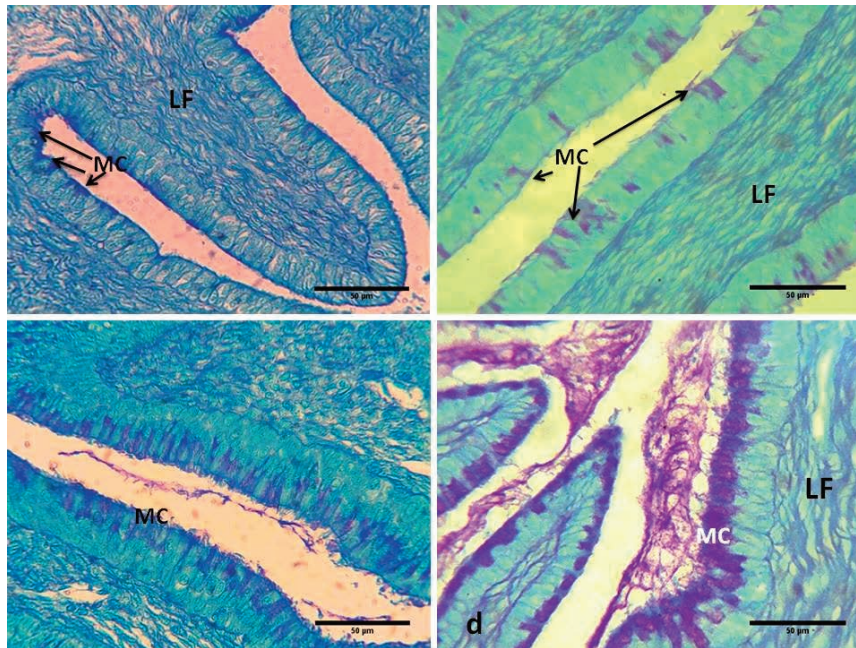


Fig. 4. The longitudinal cervical mucosal folds (LF) of the WAD goat cervixes at week 4 (a), week 8 (b), week 12 (c) and week 28 (d) showing the progressive increase in the PAS positive reactions of the epithelial cells (MC) with age. (PAS).

of the longitudinal cervical folds exhibiting PAS positive reactions (Fig. 4a). With increase in age, more cervical epithelial cells showed mucus secretory activities (Fig. 4b). At week 16 most of the epithelial cells were PAS positive (Fig. 4c) with increased secretory intensity as the animal advanced in age (Fig. 4d). External to the tunica muscularis in all age groups was the tunica serosa, a loose connective tissue framework with blood vessels.

Discussion

The length and weight of the cervix and the organo-somatic indices showed a statistically significant increase ($p < 0.05$) from birth to week 28 which was probably due to the growth and development of the cervical tissues. The cervix demonstrated openings with the uterus cranially and the vagina caudally which are communicating channels with the uterus and vagina, essential for the passage of sperm cells and foetuses during parturition. Internally the WAD goat cervix exhibited cervical rings separated by deep grooves similar to other ruminants (Habel, 1989; Dyce *et al.*, 2002; Conde-Junior *et al.*, 2012). These cervical rings and grooves probably hinder the expansion of the cervix, protect and facilitate transport of sperm cells, acts as sperm reservoir and selection of strong and viable sperm cells also reported by Halbert *et al.* (1990) and Hafez and Hafez (2000b). Even though the result showed that the number of cervical rings increased with age in this study, other authors reported more cervical rings in younger lambs than older ewes (Naqvi *et al.*, 2005; Kaabi *et al.*, 2006; Gultiken *et al.*, 2009). The interdigitating regular arrangement of the cervical rings in this study which are similar to other goats and cows, indicates a straight cervical canal that can be penetrated by an artificial insemination pipette at oestrous (Budras and Habel, 2003; Dayan *et al.*, 2010; Conde-Junior *et al.*, 2012). This arrangement contrasts with the irregularly arranged cervical rings with a tortuous canal of ewes that makes passage of artificial insemination pipette very difficult at oestrous (Habel, 1989; Kershaw *et al.*, 2005). The differences in shape, number, and arrangement of the cervical rings within ruminants were probably due to species and breed differences, animal's age, climatic factors, and nutrition (Kershaw *et*

al., 2005; Kaabi *et al.*, 2006; Conde-Junior *et al.*, 2012).

The present study revealed that the cervix of the female WAD goat at any stage of postnatal development have four layers of tissues similar to the histology of adult ruminant cervix (Aughey and Frye, 2001; Samuelson, 2007). At birth, the lamina propria-submucosa lacked glands and a less-defined smooth muscle layers suggesting that the cervix of WAD goat at birth is not well developed. This is at variance with reports that indicate the vagina, cervix and oviduct of ruminants are fully developed histologically at birth (Gray *et al.*, 2000; 2001; Carpenter *et al.*, 2003). These differences may be attributed to nutrition, geographic location climate, species or breed differences. Furthermore, the absence of cervical gland at all stages of development is similar to reports of absence of cervical glands within the walls of the cervix of adult domestic animals (Joshi *et al.*, 1976; Heydon and Adams, 1979; Banks, 1993; Samuelson 2007; Cruz-Junior *et al.*, 2014). However, cervical glands have been reported in the cervix of some domestic animals (Dellman and Carithers, 1968; Dellman and Eurell, 1998; Conde-Junior *et al.*, 2012). These differences may have been due to species, breed or environmental differences of the animals studied.

The change from a pseudostratified columnar epithelium at birth to a simple columnar epithelium by week 12 with the epithelial cells exhibiting mucus secretory activities suggests evidence of postnatal development of the cervix of WAD goat. The advanced development of the cervical epithelial cells at week 12 was similar to that described for adult ruminant cervix (Dellman and Eurell, 1998; Conde-Junior *et al.*, 2012). The initiation of mucus secretory activities of the epithelial cells in this study coincided with adenogenesis of uterine glands of WAD goats (Abiaezute *et al.*, 2017a) and may have the same inducing factors. At week 16, the cervical epithelial cell glands were fully developed with highly secretory activities and appeared similar to older WAD goats in this study.

Furthermore, the developmental stages seen in the tunica muscularis from birth was indicative of further postnatal development of the cervix similar to further development of other segments of the reproductive tracts of the WAD goat (Abiaezute and Nwaogu, 2015; Abiaezute *et al.*, 2017a, b). The description of the tunica muscularis of the cervix of the female WAD goat at week 16 was similar to that described for adult

ruminant cervix (Banks, 1993; Samuelson, 2007). The tunica muscularis in this study is an extremely dense, heavily smooth muscle-walled tissue, interspersed with dense connective tissue thus making the cervix firm and rubbery on palpation. It is this arrangement of smooth muscles interspersed with connective tissues that is believed to give the cervix its firm and inelastic properties (Halbert et al., 1990; Hafez and Hafez, 2000a; Conde-Junior et al., 2012; Cruz-Junior et al., 2014). The tunica serosa is typical and made up of loose connective tissue framework with blood vessels and lined by simple squamous epithelium (Banks, 1993; Fuchs et al., 1996).

Conclusion

The study demonstrated that the cervix of WAD goat has a straight and non-tortuous cervical canal, with cervical folds which increased with age in contrast to the ewe. Furthermore, the walls of cervix lacked glands. Rather, the epithelial cells constitute the mucus secretory cells of the cervix. The epithelial cells and the walls of the cervix at week 16 are well developed and appeared similar to cervixes of older WAD goats in this study. This indicates that the WAD goat may attain puberty at week 16, earlier than previously reported.

Conflict of interest

The authors declare that there are no conflicts of interest regarding this study.

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