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Morphological features of the uterus during postnatal development in the West African Dwarf goat (*Capra hircus*)

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Abstract

The knowledge of the age at which the West African Dwarf (WAD) goat attains puberty could be exploited to improve this breed of goat and intensify production. The objective of this study was to highlight the postnatal developmental morphology of the uterine tissues of the WAD goat from birth to 28 weeks of age. Fifty WAD goat kids were obtained and grouped into 10 groups of 5 goats per group from day old to week 28. The uterine tissues of euthanized goats were dissected out and examined grossly. Thereafter fixed uterine tissues were prepared for light microscopy and stained with haematoxylin and eosin. Histological images of the uterine tissues were captured with a moticam camera. The length and weight of the uterine horns increased significantly ($P < 0.05$) from birth to week 28 respectively. The uterine horn was shaped like a ram's horn. Internally, caruncles were prominent at birth and were regularly arranged in the uterine horn but irregularly arranged in the uterine body. Caruncles were absent in the cranial one third of the uterine horns. Histologically, the uterine horn and uterine body were similar. The endometrium was very cellular at birth without any form of glandular development, while the myometrium and the stratum vasculare were ill defined. Endometrial glands were first observed at week 1. The pseudostratified columnar epithelium of the mucosa at birth changed to simple columnar by week 12. The endometrium and the myometrium were well developed by week 12. In conclusion, the uterus of WAD goat at week 12 appeared similar to the uterus of an adult WAD goat indicating that this goat probably attains puberty earlier than the five - seven months previously reported.

Keywords: caruncles, endometrial glands, myometrium, postnatal development, West African Dwarf goat.

Introduction

The adult ruminant has a bicornuate type of uterus which is a Y shaped muscular, highly expandable tubular organ with a short body and two long tapering horns that is continuous with the uterine tubes cranially and the cervix caudally (Dyce *et al*., 2002; Budras and Habel, 2003). Internally, the uterus contains caruncles which are described as glands free while the intercaruncular areas contain large numbers of microscopic endometrial glands (Atkinson *et al*., 1984; Gray *et al*., 2001a). In synepitheliochorial type of placentation exhibited by ruminants, the caruncles are the site of implantation and fusion while the intercaruncular areas contain numerous glands that synthesize and secrete the histotroph (Amoroso, 1952; Bazer, 1975; Gray *et al*., 2001a; Igwebuike, 2009).

Mammalian uterine development is initiated in the fetus from the paramesonephric ducts. The inner mesoepithelial cells of the paramesonephric duct differentiates into the luminal and glandular epithelium, while the mesenchyme differentiates into the endometrial stroma and the myometrium of the uterus (Wiley *et al*., 1987; Kurita and Nakamura, 2008; Gonzalez and Behringer, 2009). However, mammalian uterine development is only completed during the postnatal period which establishes the three classic histological elements of the uterine wall including the endometrium, myometrium and perimetrium (Bartol *et al*., 1999; Gray *et al*., 2001a; Spencer *et al*., 2005; Cooke *et al*., 2013).

It is well known that reproduction is the primary target of economical importance in all animal production systems and early attainment of puberty is encouraged. The age of attainment of puberty in different goat breeds varies as some attain puberty between three to seven months (Michel and Rabie, 1991; Greyling, 2000; Bukar *et al*., 2006) while others attain puberty after one year (Simplicio *et al*., 1990; Al-Hosab and Basiouni, 1999). Goats are prolific breeders and the most abundant ruminant in Nigeria with an estimated population of 53.8 million, contributing about 35% of the total national meat supply and raw materials for agro-based industries (Maina, 2002; Oni, 2002; Abdel Aziz, 2010) thus contributing to the growth of the national economy. The West African Dwarf (WAD) goat, a miniature breed native to southern Nigeria has adapted to the humid tropical environment, is cheap to rear, tolerant to trypanosomosis and has high fertility and prolificacy rate (Udoh *et al*., 2010). Due to the absence of religious and cultural taboos of their products, the WAD goat meets the socioeconomic, cultural and recreational needs of the people (Devendra, 1992). It also serves as an excellent laboratory and pet animal (Taylor and Field, 2001). The WAD goat breeds throughout the year unlike temperate goats which have restricted breeding season and has been reported to attain puberty at five to seven months based on oestrous observations (Akusu *et al*., 1986; Chiboka *et al*., 1988). Numerous reproductive studies have been conducted on the WAD goat (Kirkpatrick and Akindele, 1974; Otchere and Nimo, 1975; Devendra and Burns, 1983; Akusu *et al*., 1986; Akusu, 1994; Udoh *et al*., 2010;

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Igwebuike and Ezeasor, 2013; Abiaezute and Nwaogu, 2015; Abiaezute *et al*., 2017) but none is on the developmental morphology of the uterine tissues. A large body of knowledge also exists on the prenatal and postnatal developmental morphology of the uterine tissues of other domestic animals (Desjardins and Hafs, 1969; Atkinson *et al*., 1984; Michel and Rabie, 1991; Bartol *et al*., 1993; Gray *et al*., 2001b; Bukar *et al*., 2006; Hayashi *et al*., 2008). However, these studies were on breeds of domestic animals not indigenous to the tropical climate. Moreover, most description of the goat reproductive anatomy is based on assumptions of similarities with the sheep (Smith, 1986; Adigwe and Fayemi, 2005). More also, knowledge of the age of attainment of puberty will aid to institute assisted reproductive techniques in WAD goats, whose improvement is necessary to intensify production. This will assist WAD goat producers to effectively supply a domestic and possibly external trade demand that could provide the farmers with improved source of living. Therefore the present study seeks to investigate the morphological changes that may occur in the uterine tissues from birth to week 28 in the West African Dwarf (WAD) goats.

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. Fifty 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 ten groups of five goats each including day old, week 1, week 2, week 4, week 8, week 12, week 16, week 20, week 24, and week 28 of age. Each goat was weighed and euthanized by intravenous injection of 70 mg kg-1 sodium pentobarbitone (Kyron Laboratories Ltd, Johannesburg, South Africa). The uteri were dissected out and trimmed of extraneous tissues. The length and weight of each uterine horn and uterine body were determined with a meter rule and digital weighing balance (Model AUW 120 Series Shimadzu®, Kyoto, Japan). The uteri were incised open and the internal features were examined and noted.

Histological preparation

Segments of the uterine horns and uterine body from each group were cut and fixed by immersion in Bouin`s fluid for 24 h. The segments were dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. Five 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 and studied under the Leica light microscope (Leica Galen II, Wetzlar, Germany). The thickness of the tunica muscularis of each segment of the uterine body for each group was measured using the ocular micrometer gauge calibrated with the stage micrometer gauge at 100X magnification. Images were then captured using Moticam Camera 1000 (Motic China group Ltd., Xiemen, China).

The means and standard errors (Mean ± SE) of the data obtained (length and weight of uterine horns, uterine bodies and their organ-body mass indices and the histomorphometry of the tunica muscularis of the uterine bodies) were calculated. The data were analysed by one way analysis of variance (ANOVA) and Duncan new multiple range test using SPSS (version 15.0; SPSS Inc., Chicago, USA). Significance was accepted at probability level of $P < 0.05$.

Result

Gross anatomical features

In all groups studied, the left and right uterine horns extended from the uterotubal junction with the uterine tubes and coursed caudally to meet the body of the uterus. Both horns were joined together at the caudal one third as they entered the body of the uterus by a ventral and dorsal transparent sheet, interconual ligaments. From its junction with the uterine tubes, the horn started as a very narrow tube that gradually enlarged in diameter as it coursed caudally in a spiral manner resembling a ram's horn. The body of the uterus extended from the points of entry of the paired uterine horns cranially to the firm constricted cervix caudally and represented a small portion of the entire uterus.

The length and weight of the left and right uterine horns showed statistically significant increases ($P < 0.05$) as the goats aged (Table 1). There were no statistically significant differences ($P > 0.05$) in the length and weight of the left uterine horn from that of the right horn within groups studied. Likewise, length and weight of the uterine body increased significantly ($P < 0.05$) with age (Table 1). The percentage contributions of the weights of the left and right uterine horns and the uterine body to the body weights of the goat kids showed significant differences ($P < 0.05$) with increase in age (Table 2).

Internally within the horn and body of the uterus were numerous prominent oval to quadrilateral shaped raised fleshy thickenings of the mucosa, caruncles (Fig. 1). These caruncles were regularly spaced out and longitudinally arranged in four rows in the middle and caudal thirds of the uterine horns (Fig. 2). The caruncles became irregularly arranged and less conspicuous to absent towards the tip or cranial one third of the horn. The caruncles within the body of the uterus were irregularly arranged and less numerous. Between the caruncles were the uterine mucosal folds. These arrangements were similar in all groups of female WAD goats studied. At birth, the caruncles were small and creamy in colour but became more prominent and pale to pinkish with increase in age of the goat (Fig. 1 and 2).

Table 1. Table 1 mean length (cm) and weight (g) of uterine horns and body of WAD goat during postnatal growth.

	Birth	Wk 1	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	5
		Length								
Left uterine horn	2.70 ± 0.43^{a}	3.03 ± 0.33^{ab}	3.16 ± 0.26^{ab}	3.72 ± 0.46^{b}	5.30 ± 0.93^{c}	5.50 ± 0.66^{c}	5.94 ± 0.57^{c}	7.34 ±1.18^{cd}	8.15 ± 1.50^{d}	8.34 ± 1.22^{d}
Right uterine horn	2.94 ± 0.29^{a}	3.05 ± 0.32^{a}	3.18± 0.25^{ab}	3.90 ± 0.47^{b}	5.40 ± 0.95^{c}	5.71 ± 0.78^{c}	6.09 ± 0.53^{c}	7.27 ±1.00^{cd}	8.14 ± 1.82^{cd}	8.52 ± 1.23^{d}
Uterine body	0.48 ± 0.04^{a}	0.49 ± 0.04^{a}	0.51 ± 0.04^{a}	0.60 ± 0.04^{ab}	0.73 ± 0.08^{b}	0.77 ± 0.04^{b}	1.01 ± 0.06^{c}	1.15 ± 0.09^{cd}	1.24 ± 0.08^{de}	1.41 ± 0.08^{e}
		Weight								
Left uterine horn	0.14 ± 0.03^{a}	0.16 ± 0.02 ^{a}	0.21 ± 0.02^{b}	0.49 ± 0.06^{c}	0.96 ± 0.26^{de}	0.71 ± 0.08^{d}	0.80 ± 0.10^{d}	0.84 ± 0.09^{d}	1.14 ± 0.10^{e}	1.34 ± 0.17^{e}
Right uterine horn	0.14 ± 0.03^{a}	0.16 ± 0.03^{ab}	0.21 ± 0.03^{b}	0.47 ± 0.08^{c}	0.95 ± 0.24^{de}	0.70 ± 0.08^{d}	0.80 ± 0.10^{d}	0.85 ± 0.10^{d}	1.11 ± 0.13^{e}	1.31 ± 0.18^{e}
Uterine body	0.01 ± 0.01^{a}	0.03 ± 0.01 ^{a}	0.05 ± 0.01^{ab}	0.08 ± 0.01^{b}	0.22 ± 0.02^{c}	0.25 ± 0.02^{c}	0.31 ± 0.02^{d}	0.44 ± 0.02^{e}	0.70 ± 0.03^{f}	1.01 ± 0.03^{g}

Figures with different superscripts (^{abcdef}) in a row indicate significant difference ($P \leq 0.05$).

Table 2. Organ-body mass indices of WAD goat uterine horns and body during postnatal growth.

	Birth	Wk 1	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	5
Left uterine horn	0.014 ± 0.001^{a}	0.012 ± 0.002^{a}	0.013 ± 0.002^{a}	0.022 ± 0.003^{b}	0.030 ± 0.003^{c}	0.018 ± 0.004^{a}	0.018 ± 0.004^{a}	0.012 ± 0.002^{a}	0.014 ± 0.002^{a}	0.014 ± 0.002^{a}
Right uterine horn	0.015 ± 0.003^{ab}	0.012 ± 0.002^{a}	0.014 ± 0.003^{ab}	0.020 ± 0.003^{b}	0.030 ± 0.003^{c}	0.018 ± 0.004^{ab}	0.016 ± 0.002^{ab}	0.014 ± 0.002^{a}	0.014 ± 0.002^{a}	0.014 ± 0.002^{a}
Uterine body	0.001 ± 0.0002^{a}	0.002 ± 0.0002^{a}	0.003 ± 0.0007^{b}	0.004 ± 0.0003^{b}	0.008 ± 0.002^{c}	0.007 ± 0.001^{c}	0.006 ± 0.001^{c}	0.006 ± 0.001^{c}	0.009 ± 0.002^{c}	0.010 ± 0.002^{c}

Figures with different superscripts (^{abcd}) in a row indicate significant difference ($P \leq 0.05$).

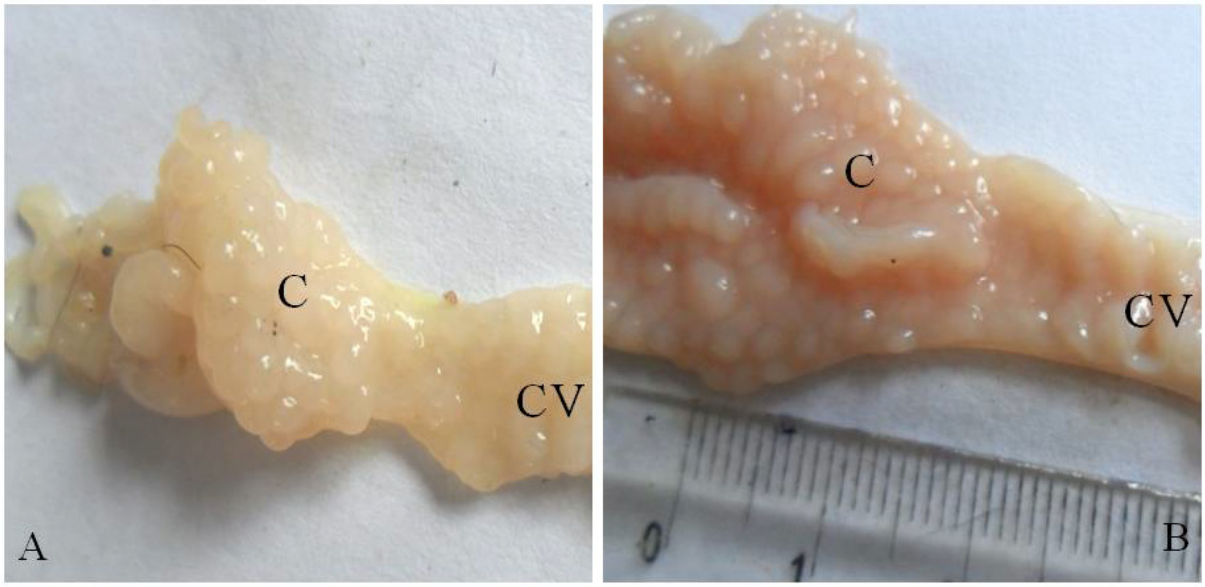

Figure 1. Gross appearance of the mucosa of the uterus of WAD goat at birth and week 12 showing the caruncles (C) and the cranial cervix (CV).

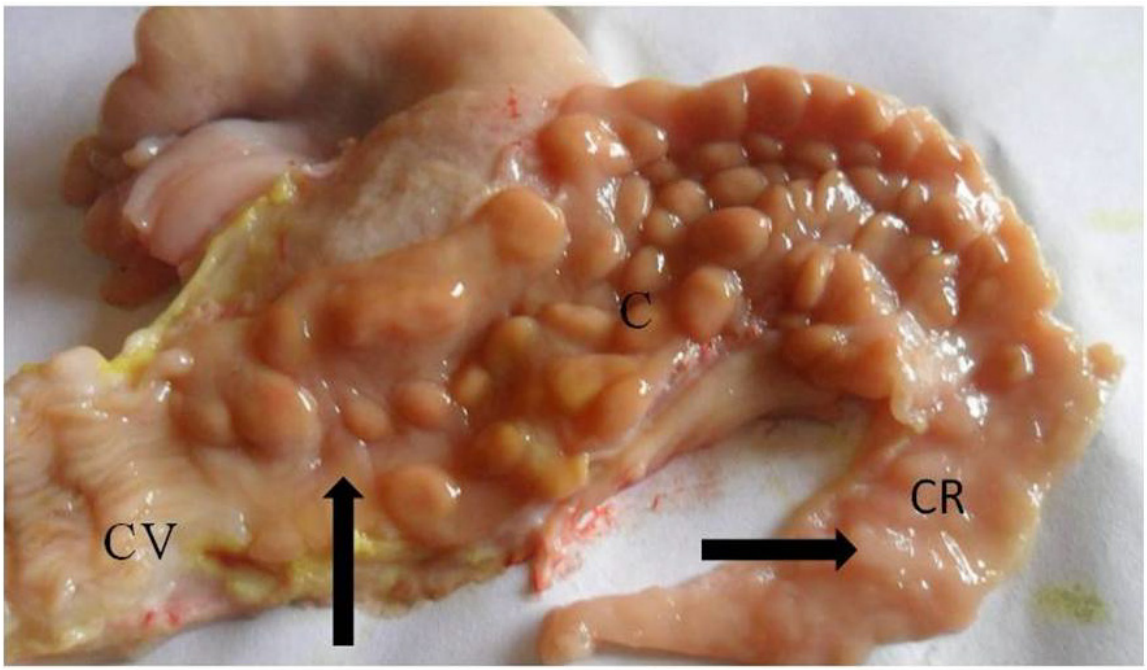

Figure 2. Gross photograph of the mucosa of the uterus of WAD goat at week 28 showing part of the cervix (CV), uterine caruncles (C) and the uterine mucosal folds (arrows). Note the absence of caruncles in the cranial one third of the uterine horn (CR).

Histology

The uterine horn and uterine body within each age group had similar histological features in the goats studied. Within their lumen projected numerous caruncles above the mucosal surface. The caruncles were separated from each other by the intercaruncular areas which were thrown into folds separated by shallow grooves (mucosal folds). In the absence of lamina muscularis mucosae, the very cellular connective tissue of the lamina propria blended with the connective tissue of the submucosa and together, extended into and formed the core of the caruncles and mucosal folds. There were no noticeable glands in the endometrium at birth and the epithelium was pseudostratified columnar. However, endometrial glands were first observed at week 1 as buds and invaginations of the luminal epithelium into the underlying connective tissues (Fig. 3). A thin cellular layer of the stratum vasculare with small blood vessels was observed within the ill defined tunica muscularis or myometrium. The perimetrium, the outer loose connective tissue or the tunica serosa was lined by a simple squamous epithelium.

Fig. 3. Cross section of the uterus of WAD goat at week 1 showing the endometrial caruncle (C), endometrium (E), blood vessel (V) of the thin stratum vasculare within the tunica muscularis (TM) and the tunica serosa (TS). Note the early forms of endometrial glands (GL).

The uterus at week 2 was similar to the uterus at week 1. However, more endometrial glands were observed within the lamina propria-submucosa of the intercaruncular area between adjacent caruncles (Fig. 4). The developing endometrial glands at this stage appeared as simple tubular glands that invaginated from the luminal epithelium into the lamina propria-submucosa. The thickness of the myometrium increased significantly ($P < 0.05$) with age (Table 3). Further development of the uterus as the goat aged involved extensive endometrial gland development which included branching and coiling within the lamina propria-submucosa. Also, the stratum vasculare and the myometrium increased in size as the goat aged and the blood vessels became larger and more numerous. At week 12, the uterus was at a very advanced stage of development with well defined caruncles, numerous endometrial glands all over the lamina propria-submucosa except within the caruncles (Fig. 5A). The myometrium was well developed with an inner circular and outer longitudinal smooth muscle layers. Also within the tunica muscularis, the stratum vasculare was well defined with numerous blood vessels. The uteri of the weeks 16-28 old female WAD goats were very similar to the uterus of the week 12 old female WAD goats (Fig. 5B). The epithelial lining of the mucosa transited from pseudostratified columnar epithelium at birth (Fig. 6A) to a complete simple columnar epithelium at week 12 (Fig. 6B).

Fig. 4. Photomicrograph of the uterus of WAD goat at week 2 showing early forms of blood vessels (V) within the caruncle (C), tunica muscularis (TM) and tunica serosa (TS). Note the glands (GL) at the intercaruncular area. H & E.

Table 3. Histomorphometry of the tunica muscularis of the uterine body of WAD goat during postnatal growth.

	Birth	Wk 1	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	5
Tunica muscularis	10.90 ± 0.94^{a}	11.50 ± 1.1^{a}	19.10 ± 2.76^{b}	28.30 ± 4.67^{c}	38.90 ± 3.80^{d}	48.30 ± 5.05^{e}	56.50 ± 6.38^{ef}	65.20 ± 4.41^{f}	72.30 ± 4.40^{fg}	79.10 ± 4.23^{g}

Figures with different superscripts (^{abcd}) in a row indicate significant difference ($P \leq 0.05$).

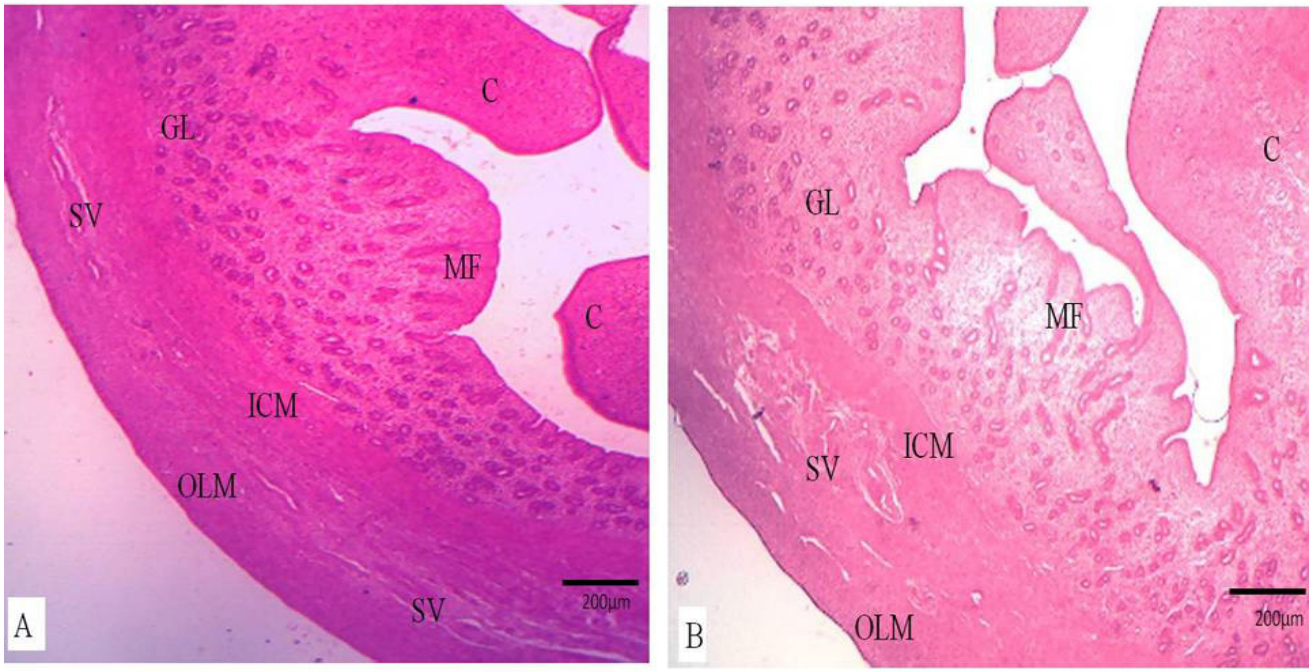

Figure 5. Cross section of the uterus of WAD goat at week 12 (A) and week 24 (B) showing well developed stratum vasculare (SV) between the outer longitudinal (OLM) and inner circular (ICM) smooth muscle layers. Note the caruncle (C), mucosal folds (MF) and the numerous glands (GL) within the endometrium. H & E

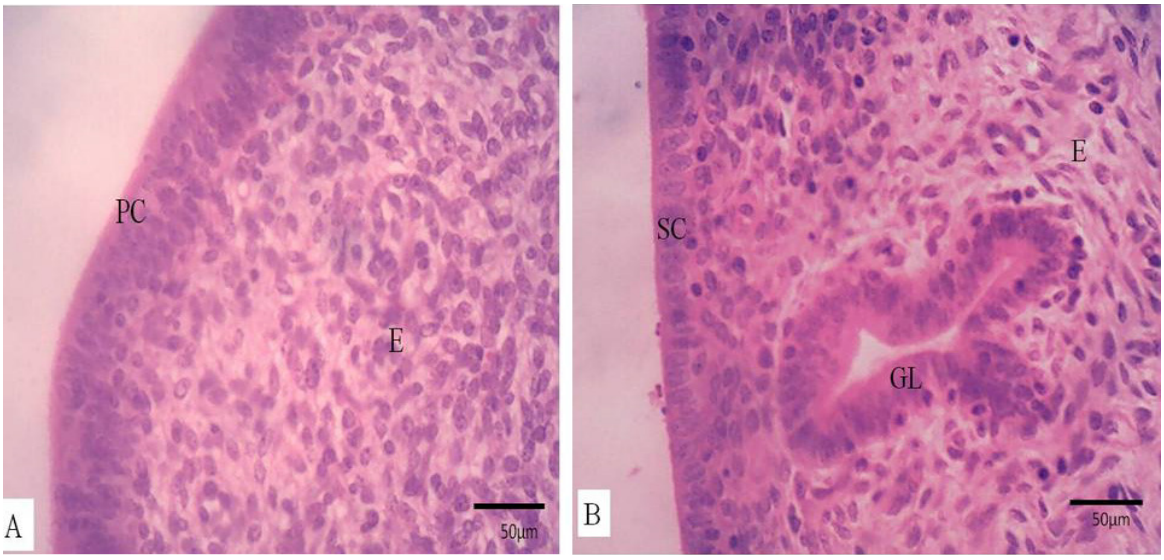

Figure 6. Photomicrograph of the epithelium of the uterus of WAD goat at birth (A) and week 12 showing the pseudostratified columnar epithelium (PC) and the simple columnar epithelium (SC). Note the endometrial gland (GL) in the cellular endometrium (E) at week 12. H & E.

Discussion

This study provides information on the gross and microscopic features of the uterus of WAD goat from birth to week 28. Grossly, the uteri of the WAD goat in all age groups studied were similar. However, there was a statistically significant increase ($P < 0.05$) in length and weight as the animal aged. This pattern of uterine growth was similar to that observed in small ruminants (Kennedy *et al*., 1974; Bukar *et al*., 2006; Hayashi *et al*., 2008). These could be due to increased development of the uterine tissues occasioned by the increase in the numbers and activities of the endometrial glands and the proliferation of the cellular components of the uterine wall.

The numerous prominent raised caruncles in the uteri of the WAD goat at birth which were reportedly not prominent in Sahel goats at birth (Sivachelvan *et al*., 2002; Bukar *et al*., 2006) suggest prenatal caruncular morphogenesis. Similar observations have been reported in ewe lambs at birth (Wiley *et al*., 1987; Bartol *et al*., 1999; Hayashi *et al*., 2008). This study demonstrated the absence of caruncles in the cranial one third of the uterine horns in WAD goats of all ages. This is at variance with report of caruncles observed in all parts of the uterine horn including the tips of the horns in ewe and cow (Stevens *et al*., 1981; Habel, 1989; Dyce *et al*., 2002) and goats (Lyngest, 1968). This arrangement in WAD goat also reported in adult goats in Egypt (Abd-Elnaeim, 2008) could be an adaptation to prevent implantation of foetuses in the small, narrow and spiral shaped cranial portion of the uterine horn.

The quadrilateral shaped caruncles in this study appeared as raised fleshy thickening of the mucosa similar to that reported by Abd-Elnaeim (2008) in adult goats in Egypt, thus suggesting non-stalked caruncles that are closer to the mucosal surface of the uterus This differed from the round to oval gently sloping hillock-like caruncles in sheep (Stevens *et al*., 1981) and mushroom or dome shaped caruncles with stalks in bovine (Pfarrer *et al*., 2001; Dyce *et al*., 2002; Budras and Habel, 2003). These non stalked quadrilateral caruncles in WAD goats probably favoured firmer attachment of the placenta to the uterine wall.

Histologically, the uterus of WAD goat showed a marked postnatal uterine morphogenesis similar to the uterine development of ewe (Kennedy *et al*., 1974; Bartol *et al*., 1988a; Hayashi *et al*., 2008), Syrian goat (Michel and Rabie, 1991) and Sahelian goat (Bukar *et al*., 2006) from birth to puberty. The postnatal events observed included the emergence and development of endometrial glands, development of endometrial stroma and the growth of the myometrium. The timing of these developmental events of the uterus is specie specific and reflects differences in uterine maturity at birth (Gray *et al*., 2001a). It may also be associated with the´´programming'' of uterine tissues for productivity in adult life (Kobayashi and Behringer, 2003; Bartol *et al*., 2008).

The developmental pattern of endometrial gland in this study was similar to the developmental pattern of endometrial glands in ewe (Kennedy *et al*., 1974; Wiley *et al*., 1987; Bartol *et al*., 1988b; Gray *et al*., 2001b; Hayashi *et al*., 2008) where endometrial gland development was initiated between day 0 and day 7. However, this pattern differed from that reported in Sahel goat kid where glandular development was first noticed from the second week (Bukar *et al*., 2006). In this study, the advanced development of uterine glands in the endometrium at week 12 suggests a uterus that appeared similar to that described for an adult small ruminant (Wiley *et al*., 1987; Shalini and Sharma, 2004; Samuelson, 2007; Abd-Elnaeim, 2008; Katare *et al*., 2015). The increased development with age of blood vessels within the caruncles and endometrial glands within the intercaruncular areas suggest the evolutionary adaptation of the WAD goat to its synepitheliochorial type of plancentation.

The growth and development of the

myometrium and the stratum vasculare from birth to week 28 in this study suggests immaturity of these tissues at birth thus requiring further postnatal growth and development for productivity at puberty. The values of the myometrium in this study are lower than the values reported in prepubertal Indian goats (Singh and Prakash, 1990) and Gaddi goats (Shalini and Sharma, 2004). This variation may be due to the age, larger size and weight at attainment of puberty in these female goats. However, the morphology of the myometrium and the stratum vasculare at week 12 in this study were similar to that described for adult small ruminants (Wiley *et al*., 1987; Singh and Prakash, 1990; Banks, 1993; Shalini and Sharma, 2004; Samuelson, 2007; Katare *et al*., 2015). Further postnatal development of the myometrium may be related to their functions (Wimsat, 1950; Stewart *et al*., 2000; Gray *et al*., 2001b).

The patterns of development observed in this study agrees with reports that establishment of tissue-specific histoarchitecture of the uterus is only completed postnatally (Gray *et al*., 2001a; Spencer *et al*., 2005, 2012). Thus, it may be inferred that the uterus of WAD goat at week 12 of age may be similar to that of an adult WAD goat. This observation may indicate early attainment of puberty in WAD goats. Although Akusu *et al*. (1986) and Chiboka *et al*. (1988) reported first oestrus in WAD goats to occur between 5-7 months of age, more recent authors have shown that the goat may attain puberty as early as 3-4 months of age (Jainudeen *et al*., 2000; Khanum *et al*., 2000; Bukar *et al*., 2006; Abiaezute *et al*., 2017).

The present study has demonstrated the absence of caruncles in the cranial one third of the uterine horn. The study showed postnatal developments that established the three classic histological elements of the uterine wall (endometrium, myometrium and perimetrium) and the process of adenogenesis. More also, the WAD goat probably attains puberty at three to four months which is earlier than previously reported and this could be used to improve productivity in this breed.

Conflict of interest

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

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