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Pregnancy Related Biometrical and Histological Changes in the Dromedary Ovaries and Uterus

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With 12 figures, 1 table and 26 references

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ABSTRACT

Background: Biometrical and histological studies on pregnancy related changes in the ovaries and uterus of the one-humped camel (*Camelus dromedarius*) were undertaken in Maiduguri, Borno State, Nigeria, using abattoir samples, with the objective of documenting information on these aspects.

Methods: The 13 months dromedary gestation period was divided into four quarters for the purpose of these studies and a total of 44 pregnant and 11 non-pregnant adult dromedary ovaries and uteri were used. Biometrics and photographs of the organs were taken before their samples were fixed in 10% formal saline for histological studies.

Results: Grossly, the left ovaries were significantly larger [$p < 0.05$] than the right both in pregnant and non-pregnant states, suggestive of their natural activity in the dromedary. Corpus luteum was mostly (98%) found in the left ovaries throughout pregnancy and occasionally in non-pregnant states. The uterine horns and body showed progressive significant increase in size with advancing pregnancy as a result of pregnancy related increase in size of the foetus, its membranes and volume of the associated fluids. Pregnancy caused reduction in length and increase in the diameter of the external sphincter muscle of the cervix but does not seem to have significant effect on the internal cervical sphincter muscle. Thus, stressing the usefulness of contraction of the external cervical sphincter muscles in the sealing of the cervix during pregnancy. Histologically, the non-pregnant ovarian cortex showed different developmental stages of ovarian follicles, atretic follicles and, rarely, corpus luteum while the medulla was highly vascular. The corpora lutea from the gravid and non-gravid dromedary cows were characterized by granulosa lutein and theca lutein cell types amidst stromal connective tissues and vessels. The granulosa lutein cells constituted the majority of the observed lutein cells. The dromedary placenta was observed to be of the diffused, villous epitheliochorial type with a histotrophic mode of nutrition. It was characterized by areola spaces and multinucleated chorion giant cells during the first two quarters of gestation. The areola spaces disappeared towards the third quarter.

Conclusion: It was concluded that the gross features of the dromedary uterus are similar to those of the horse and that the histology of its placenta is similar to those of the sow.

Key words: Biometry, dromedary, histology, ovary, placentation, pregnancy, uterus

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Introduction

Camels appear to have made remarkable comeback in recent years. Somewhat neglected until the start of the 1980s, there has been increasing interest by scientists in their ability to survive and reproduce in harsh and hostile environments and by development workers in their roles as life support systems in these environments [Wilson, 1997].

The dromedary, one-humped camel, ovaries are lobulated, reddish brown in colour, oval or circular in shape. Each ovary weighs between 5 and 15 g. The size and weight depend to some extent on the activity. Non-functioning ovaries may weigh about 3.7 g, those with corpus luteum (CL) of pregnancy about 8 g. Each ovary is enclosed in a long conical pocket-like fold of mesosalpinx called the bursa ovarii. The apex of this bursa forms a large circular orifice within which lie the fimbriae of the oviduct [Tayeb, 1953; Shalash and Nawito, 1964; Shehata, 1964; Charasbi *et al.*, 1975; Musa and AbuSineina, 1978].

The non-gravid uterus of an adult dromedary is reddish white, shiny and smooth with a short body of only 2 - 3.5 cm in length and the horns vary between 6 - 10 cm (right) and 8 - 15 cm (left). Its two horns are closely separated by a median septum internally. Its cranial end is marked by what is almost a T-junction [Joshi *et al.*, 1978]. The size, tone and location of the uterine horns change throughout pregnancy. The left uterine horn, in which the foetus implants, becomes noticeably distended at around one and a half month of pregnancy and is almost double its size by two months. By 150 days, the diameters of the gravid and non-gravid horns are nearly four times their original sizes. The placenta in dromedary is diffused epitheliochorial in nature, without cotyledons [Khanna *et al.*, 1987].

Information on the biometry and histology of the dromedary reproductive organs is rare, even rarer are those related to pregnancy. This present study aims at documenting information on the biometrical and histological changes in the ovaries and uterus during the four quarters of the dromedary gestation.

Materials and Methods

The study was carried out on the left and right ovaries and the uteri of forty four pregnant and eleven non-pregnant, apparently healthy adult dromedaries of varying ages and weights procured from the Maiduguri Metropolitan abattoir, Maiduguri, Nigeria. The thirteen months dromedary gestation period was divided into four quarters for the purpose of this study [Jaji *et al.*, in press]. Scalpels were used to incise, excise, separate and debride the organs. 100% spun polyester sewing thread (Two Birds Brand®) and a superior tailoring rule (Butterfly Brand®) were used to measure lengths of the ovaries and uteri.

The length of each ovary was the distance between its cranial and caudal poles; the thickness was the distance between its attached and free borders and the diameter the distance between its medial and lateral surfaces.

The length of each uterine horn was the distance from the middle of the point of uterine bifurcation (T-junction) to its tip and the breadth was the greatest distance between its left and right walls. The length of the uterine body was the distance from the point of uterine bifurcation to the tip of internal cervical os and its breadth was the greatest distance between its left and right walls. The lengths of the internal and external parts of the cervical canal were the individual distances between the tips of each part to the raised point of the cervical canal; their breadths were the greatest distances of their left and right walls.

The differences between the above dimensions in pregnant and non - pregnant dromedaries were tested using the one-way analysis of variance (ANOVA) from the computer statistical software, Graph pad Instat® version 3.06, 32 bit for Windows.

Specimens of ovaries from the different quarters of gestation were fixed in 10% formalin for histological slide production. They were embedded in paraffin and sectioned at 5 - 7 μ m thickness. These sections were later stained with haematoxylin and eosin (H & E) [Luna, 1968; Baker and Silver, 1979]. The produced histological slides were observed under light microscopy using Olympus VAN-OX research microscope, for pregnancy related histological changes.

Results

Gross changes

Ovaries

Grossly, in the non-pregnant dromedaries, the ovaries were observed to be reddish in colour and flat in shape. The two surfaces of the ovaries had ovisacs that gave them a grape bunch appearance (Fig. 1). In the pregnant dromedaries however, the corpora lutea were observed. The corpora lutea had flabby to firm consistency along the quarters of gestation.

In the adult non pregnant dromedaries, the left ovary measured 3.35 ± 0.62 cm in length, 2.46 ± 0.41 cm in diameter, 1.23 ± 0.19 cm in thickness and 4.32 ± 0.50 g in weight while the right measured 2.61 ± 0.44 cm in length,

1.72 ± 0.23 cm in diameter, and 0.91 ± 0.41 cm in thickness and 3.39 ± 0.45 g in weight. 98% of the pregnant camels studied had CL in their left ovaries. The biometrics of pregnant and non - pregnant ovaries of the present study are presented in Table 1. Most of these dimensions showed significant increases as pregnancy progresses.

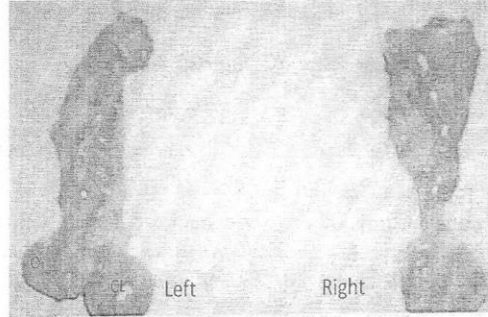


Fig. 1. Photograph of the left and right tubes with the ovaries of a 3 - 4 months pregnant dromedary. Note the corpus luteum (CL) on the left ovary (O)

Table 1. Biometrics of reproductive tract of pregnant and non-pregnant dromedaries in Maiduguri, Nigeria

Organ		Dimensions	Non-pregnant	Physiologic state of dromedary			
				Pregnant			
				2-4 months (1 st quarter) of pregnancy	4-7 months (2 nd quarter) of pregnancy	7-10 months (3 rd quarter) of pregnancy	10-13 months (4 th quarter) of pregnancy
Ovary	Left	Length (cm)	3.35±0.62	3.98±0.19***	4.46±0.18***	4.92±0.20***	5.48±0.17***
		Diameter (cm)	2.46±0.41	2.41±0.07 ^{ns}	2.59±0.12 ^{ns}	2.79±0.08***	2.90±0.16***
		Thickness (cm)	1.23±0.13	.20±0.05 ^{ns}	1.29±0.06 ^{ns}	1.41±0.10**	1.41±0.16**
		Weight (g)	4.32±0.50	10.86±0.16***	10.96±0.40***	11.32±0.08***	11.54±0.09***
	Right	Length (cm)	2.61±0.44	2.70±0.11 ^{ns}	3.07±0.12***	3.36±0.10***	3.59±0.11***
		Diameter (cm)	1.72±0.23	1.97±0.12**	2.26±0.11***	2.56±0.08***	2.77±0.14***
		Thickness (cm)	0.91±0.14	0.78±0.05**	0.89±0.05 ^{ns}	1.01±0.06*	1.17±0.06***
		Weight (g)	3.39±0.45	4.89±0.16***	5.31±0.13***	5.70±0.09***	6.10±0.16***
Uterine horn	Left	Length (cm)	11.47±2.15	37.19±3.55***	41.97±1.03***	45.28±1.72***	47.36±1.13***
		Diameter (cm)	4.54±1.48	14.65±0.43***	15.82±0.56***	17.65±0.71***	18.30±0.53***
	Right	Length (cm)	7.06±1.60	12.66±1.42***	13.50±0.90***	16.23±0.73***	17.36±0.62***
		Diameter (cm)	3.55±1.01	7.65±0.77***	8.55±0.23***	8.86±0.36***	9.50±0.37***
Uterine body		Length (cm)	7.97±1.35	34.86±2.64***	37.40±2.61***	44.59±2.48***	47.86±1.98***
		Diameter (cm)	4.36±0.96	14.16±2.23***	17.84±1.23***	20.96±1.51***	23.63±1.18***
Cervix	External os	Length (cm)	4.56±0.70	1.40±0.31***	0.94±0.55***	1.26±0.44***	1.43±0.42***
		Diameter (cm)	1.04±0.32	1.71±0.20***	1.54±0.25***	1.66±0.31***	1.73±0.27***
	Internal os	Length (cm)	2.01±0.34	2.39±0.29 ^{ns}	1.86±0.46 ^{ns}	2.35±0.44 ^{ns}	2.38±0.45 ^{ns}
		Diameter (cm)	0.76±0.19	0.68±0.17 ^{ns}	0.71±0.15 ^{ns}	0.72±0.16 ^{ns}	0.75±0.13 ^{ns}

Figures are mean ± SD; * = significant at 0.05; ** = very significant at 0.01; *** = extremely significant at 0.001; ^{ns} = not significant

Uterine horns

In the non-pregnant dromedaries, the left uterine horn (Fig. 2) measured 11.47 ± 2.15 cm in length and 4.54 ± 1.48 cm in diameter while the right measured 7.06 ± 1.60 cm in length and 3.55 ± 1.01 cm in diameter. The biometrics for pregnant and non-pregnant uterine horns of the present study are presented in Table 1. Most of these dimensions showed significant increases as pregnancy progresses.

Uterine body

In the non-pregnant dromedaries, the uterine body (Fig. 2) measured 7.97 ± 1.35 cm in length and 4.36 ± 0.96 cm in diameter. The biometrics of the pregnant and non-pregnant dromedary uterine bodies of the present study are presented in Table 1. Most of these dimensions showed significant increases as pregnancy progresses.



Fig. 2. Photograph of the reproductive tract of a non-pregnant dromedary showing the locations of its ovaries (O), oviducts (Ov), uterine horns (UH), uterine body (UB), cervix (C), vagina (V) and broad ligament (BL). Note: the cervix has a central raised part (*) that separates the cervical canal into internal (I) and external (E) parts

Cervix

In the non-pregnant dromedary, the internal part of the cervical canal measured 2.01 ± 0.34 cm in length and 0.76 ± 0.19 cm in diameter while the external measured 4.56 ± 0.70 cm in length and 1.04 ± 0.32 cm in diameter. The biometrics of the pregnant and non-pregnant cervical canals are presented in Table 1. Most of these dimensions also showed significant increases as pregnancy progresses.

Histological changes

Histology of the non-pregnant dromedary ovary

Histologically, the left ovary of the non-pregnant adult dromedaries was observed to comprise a broad peripheral zone, the cortex and an inner area, the medulla. The cortex had different developmental stages of ovarian follicles, corpus haemorrhagicum and atretic follicles (Figs. 3, 4 and 5a and b) while the medulla had many large coiled blood vessels (Fig. 6).

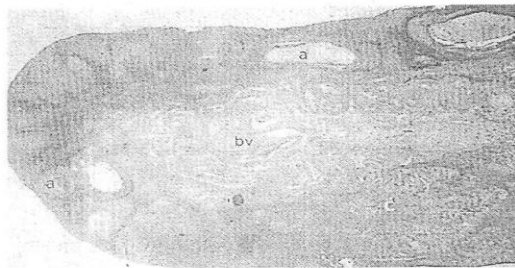


Fig. 3. Micrograph of a non-pregnant dromedary ovary depicting the arrangement of its cortex and medulla. Corpus haemorrhagicum (C) and atretic follicles (a) are in the cortex and the medulla had net-work of coiled large blood vessels (bv). H & E $\times 20$

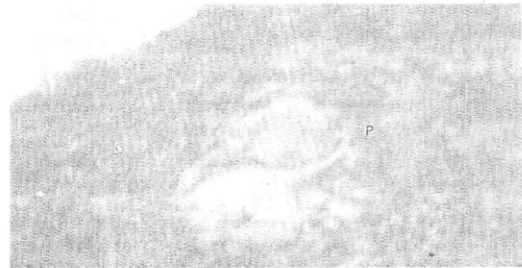


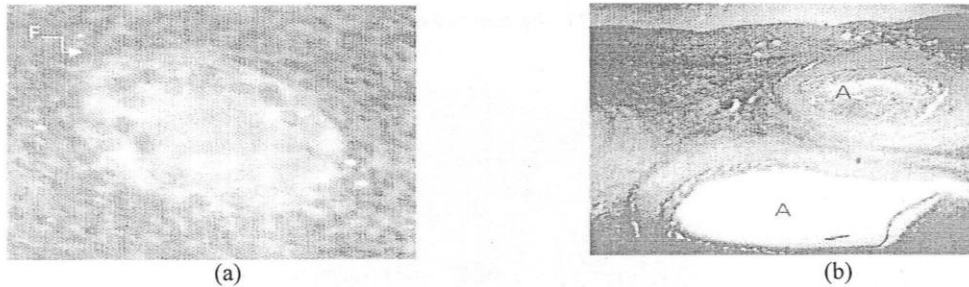
Fig. 4. Micrograph of a non-pregnant dromedary ovarian cortex depicting stromal cells (S), promordial (P) and unilaminar primary (U) follicles. Note: the primordal follicle is composed of a primary oocyte surrounded by a layer of squamous follicular cells while the unilaminar follicle is composed of a primary oocyte surrounded by a layer of cuboidal follicular cells. H & E $\times 40$

Histological changes in the pregnant dromedary ovary

In addition to the above stated features of the non-pregnant dromedary ovaries, the pregnant dromedary ovary was characterized with CL. Throughout pregnancy, the left ovary was the most active ovary, 98% of CL observed in the present study was in the left ovaries. Large [granulosa] lutein cells and small (theca) lutein cells were observed in the corpus luteum. The large lutein cells were more than the small lutein cells. Many blood vessels were also found in the luteal stroma (Figs. 7a and b).

Histology of the normal non-pregnant dromedary uterus

The mucosa had some elevated areas bulging into the lumen (Fig. 8). Lamina epithelialis of the mucosa was made up of simple columnar epithelium while the lamina propria blends with the submucosa to form propria-submucosa without an intervening lamina muscularis. The propria-submucosa had glands, blood vessels and nerves. The tunica muscularis was highly muscular with spirally arranged muscles in areas associated with mucosal elevations and an inner longitudinal outer circular arrangement of muscles in areas not associated with mucosal elevations. The connective tissue of the serosa was of the loose type and has smooth muscle fibers and blood vessels.



Figs. 5a and b. Micrographs of a non-pregnant dromedary ovarian cortex depicting (a) a multilaminar primary follicle composed of a primary oocyte surrounded by a double layer of cuboidal follicular cells (F), and (b) atretic follicles (A). H & E $\times 100$

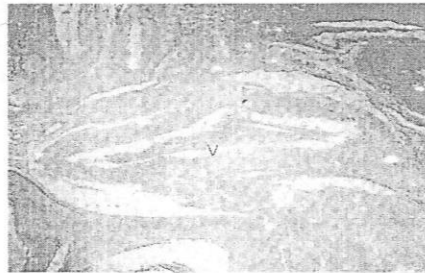


Fig. 6. Micrograph of a non-pregnant dromedary ovarian medulla depicting its network of coiled large blood vessels (V). H & E $\times 100$

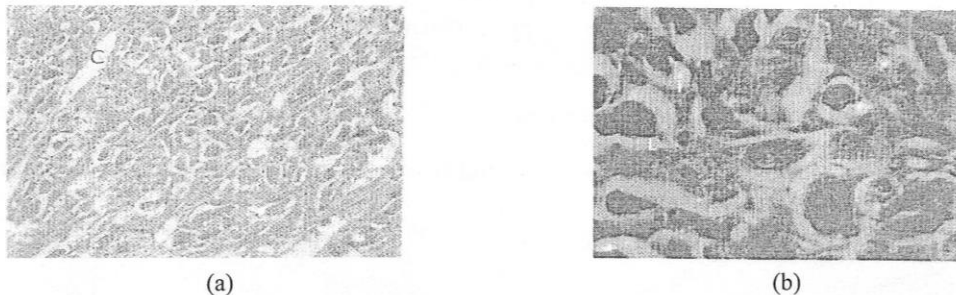


Fig. 7a and b. Micrographs of the corpus luteum of the left ovary of a dromedary in second quarter of gestation. Note the large (granulosa) lutein cells (L) are many while the smaller (theca) lutein cells (I) are few. Blood capillaries (C) are also observed. H & E (a) $\times 100$ (b) $\times 200$

Histological changes in the pregnant dromedary uterus

First quarter of gestation

The foetal chorion started formation and its villi were observed to associate with the crypts of the maternal endometrium. The chorion was fairly vascularized and maternal endometrial glands activity had started. The chorion had loose connective tissues and areolar spaces separate it from the areas of the chorion leave at this quarter (Figs. 9 and 10).

Second quarter of gestation

The foetal chorion increased the vascularization and stratification of its cuboidal epithelium. The epithelium of the chorion along with the adjoining mesenchymal tissue formed networks around the partitioned areola spaces that are now well formed. The areolar spaces were located opposite the openings of endometrial glands that have now increased formation. Nutrients seem to be diffusing from the endometrial glands into the areolar spaces for absorption by multinucleated giant cells (Fig. 11) of the foetal villous chorion. The maternal simple low columnar epithelium and the stratified cuboidal epithelium of the foetal chorion were intact.

Third quarter of gestation

The chorion mass has reduced in size and the villus-crypt association became more intimate, the two came into closer contact to form branches and anastomoses. The foetal villi and maternal crypts were highly vascularized and separated by connective tissues and epithelial linings. The areolar spaces have greatly reduced in size, thereby making

the placental components more obvious (Fig. 12). The maternal endometrial glands have immensely increased in size and covered more areas of the endometrium.

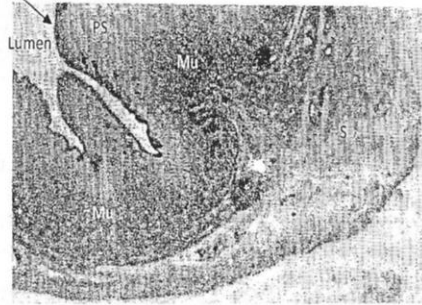


Fig. 8. Micrograph of a portion of the left uterine horn of an adult non-pregnant dromedary showing the organization of its lamina epithelialis (arrow), propria-submucosa (PS), tunica muscularis (Mu) and tunica serosa (S). H & E $\times 40$



Fig. 9. Micrograph of the placentation of a dromedary in the first quarter of gestation. Note it has a diffused villous type of placentation (P) made by the contribution of foetal chorion and maternal endometrium; the maternal endometrium has started its gland (G) formation and the muscularis (M) was predominantly made of circular muscles. H & E $\times 20$



(a)



(b)

Fig. 10a and b. Micrographs of the placentation of a dromedary in the first quarter of gestation. Note: the chorion (C) with loose connective tissues, scanty vessels (V) and its villi (V) are observed to associate with the maternal crypts (M). The villi and crypts are separated by areolar spaces (*) that contain histotrophs (H). Endometrial glands (E) are also seen. H & E $\times 100$

Fourth quarter of gestation

The epithelium of the fetal chorionic villi and maternal crypts were closest at this stage.

Discussion

Gross changes

The left and right ovaries of the non-pregnant dromedaries were observed as reddish, flat organs with grape bunch-like appearance and their dimensions and weights were similar to those reported by Wilson [1984], Ribadu

[1988], Umaru and Mera [2000] with slight variations. At early stage of pregnancy, oval, flabby CL, which is normally observed during pregnancy in the camel [Musa and AbuSineina, 1978], was observed in 98% of the left ovaries. According to Wilson [1984], the left ovary of the dromedary is apparently more active than the right and a very high percentage of pregnancy occur in the left horn of the uterus. In a study on Sudanese breeds of dromedary 98.6% [Musa and AbuSineina, 1976] and in another study on Egyptian breeds 99.24% [Shalash and Nawito, 1964] CL were all recorded at the left horn. The CL became larger and firmer with advancing pregnancies, in conformity with earlier observation by Shalash [1965]. The establishments and further increases in size of the CL across the four quarters of gestation were associated with attendant increases in the overall size and weight of the ovaries. The CL persisted in the ovaries throughout the gestation in agreement with earlier observations by Wilson [1984]. Umaru and Mera [2000] gave a breed related comparatively slightly lower dimensions and weights of the left ovaries of the non-pregnant, and slightly higher dimensions and weights of the left and right ovaries of the pregnant dromedaries in north western Nigeria.

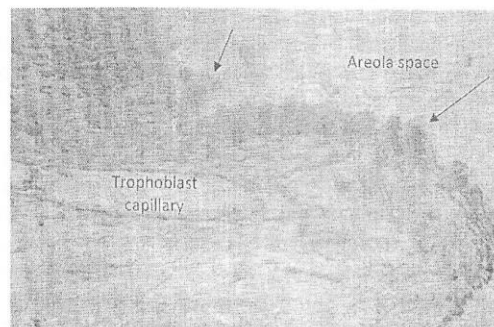


Fig. 11. Multinucleated chorion giant cells (arrows) of the chorion of a second quarter dromedary fetus. H & E $\times 400$

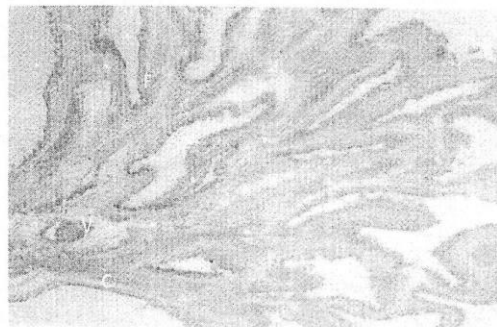


Fig. 12. Branching and anastomoses of foetal villi (F) and maternal crypts (C) that characterized the third quarter of dromedary gestation. The villi and crypts are vascularized (V) and the areolar spaces are greatly reduced in size. H & E $\times 100$

The present study was able to reaffirm earlier statement by Joshi *et al.* [1978] that though the dromedary is a ruminant, the gross features of the reproductive tract of the female have some resemblance with those of the horse.

The left uterine horn was larger than the right uterine horn both in pregnant and non-pregnant states and majority of pregnancies in the present study were on the left uterine horn. The length and diameter of the non-pregnant uterine body in this study are higher than the ranges given by Shalash [1965] and Ribadu [1988] but slightly lower than those of Umaru and Mera [2000]. These differences could also be attributed to variations in breed. Just like the uterine horn, there were dramatic increases in size of the uterine bodies with advancing pregnancies. According to Wilson [1984] the size of the uterine horns and body show marked increases during the stages of gestation. The length of the uterine body of the present study at full term was significantly higher than those of non-pregnant. The increases in size were attributed to increasing foetal size and volume of uterine secretions associated with advancing pregnancies.

The mean dimensions of the whole cervical canal in non-pregnant dromedaries of the present study were similar to earlier reported dimensions by Shalash [1965], Mukasa-Mugerwa [1981], Ribadu [1988], Umaru and Mera [2000]. The lengths of the external part of the cervical canal showed significant reductions, while its diameter significantly increased, throughout the course of gestation. This could be attributed to the contraction of the sphincter muscles of this part. The contraction of the external sphincter muscle is the most useful in the sealing of the cervix during pregnancy due to its proximity to the exterior. On the other hand, pregnancy does not seem to have much effect on the dimension of the internal part of the cervical canal, in agreement with the dimensions recorded by Umaru and Mera [2000].

Histological changes

In the non-pregnant dromedary, the ovarian cortex was marked with primordial, growing and Graafian follicles. Corpus haemorrhagicum and corpus luteum (CL) were occasionally seen in some ovaries. The CL might have resulted from pseudo-pregnancies or early embryonic deaths as regressed CL [Wilson, 1984]. The medulla was vascular. During pregnancy, the ovary was characterized by the CL, which consisted of large and small lutein cells. The large [granulosa] lutein cells constituted the majority of the luteal cells and are derived from the preovulatory granulosa layer of the parent Graafian follicle, while the small [theca] lutein cells were few and are derived from the theca interna of the preovulatory parent Graafian follicle [Dellmann and Eurell, 1998]. Many blood vessels and connective tissues were also found in the luteal stroma.

The present study observed that the dromedary placenta was of the diffuse villous epitheliochorial type, further confirming earlier reports by Wilson [1984] and that it appears to resemble most closely that of pig in its formation of areolar spaces during the first half of gestation [Amoroso, 1952]. The chorion started its formation, stratification, vascularization and areola formation at the early parts of first quarter of gestation. Occasionally, giant [multinucleate] cells were evident in the chorion during the first quarter of gestation. The camel chorionic trophoblast giant cells are unlike the large, uniformly binucleated placental lactogen secreting cells described of ruminants, which fuse with endometrial epithelial cells to form syncytial plaques [Wooding, 1982, 1983]. Nor are they like the uniformly binucleated invasive trophoblast cells of the equine chorionic girdle [Allen and Moor, 1972] or the various cells with single enormous nuclei that are found in the placenta of some rodents [Deane *et al.*, 1962]. The camel giant cells remain localized to the chorion and do not fuse with or migrate into maternal tissue.

The chorion increased its stratification and vascularization by the second quarter of gestation, during which there were more invaginations of the endometrial epithelium to form crypts with which foetal chorion villi associated. The areolar spaces formed by the chorion increased in size and were located opposite to the openings of the endometrial glands. Nutrients seem to be collecting in the areolar spaces, from where they were absorbed by the trophoblast giant cells that have now increased in number, thus suggesting a histotrophic mode of nutrition. Although, there are controversies on the true functions of the trophoblast giant cells [Deane *et al.*, 1962; Soares, 1987; Yamaguchi *et al.*, 1992], their location at the mouth of endometrial glands, suggests their likely absorptive function.

By the third quarter of gestation the crypt-villi association and trophoblast vascularization were more intense. These were associated with reductions in chorion epithelial stratification, size and frequency of areolar spaces and population of the trophoblast giant cells. According to van Lennep [1961] the chorion giant cells are present, although reduced in number, during the second half of pregnancy.

The areolar spaces and associated chorion giant cells population were greatly reduced by full term.

Based on this study, it was concluded that the gross features of the dromedary uterus are similar to those of the horse and that the histology of its epitheliochorial placenta is similar to those of the sow.

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