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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(4): 3629-3639. **OPEN ACCESS**

Anatomical, histological and in vitro study debating some reasons for left horn pregnancy phenomenon in the one-humped she-camel(camelus dromedarius)

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The present study was carried out on arterial supply of uterus, histological studies on horns and In-vitro production of the camel embryo. The study aimed to give an attention for the left horn pregnancy phenomenon reasons and to study the effect of adding oviductal extracts on the culture medium on the developmental competence on dromedary camel oocytes. Study conducted on twenty non-pregnant uteri of one-humped she-camel aged 8 – 11 years old during breeding and non-breeding seasons. Major blood vessels were described and its measurements were statistically analyzed. The record showing a significant increase in the thickness of the blood vessels diameter in the left side than those supplying the right side of the uterus. Three different stains were used, H & E stain, Masson's Trichrome and PAS technique. The results obtained showed the endometrial thickness of the breeding right horn was measure higher than the left one. Moreover, the difference in the quantification of endometrial glands were represent higher % of the mucosa in right than left horn. For the in-vitro application, following IVC of fertilized oocytes using either right or left oviductal fluid extract in breeding and non-breeding seasons. the results revealed that, a significant increase in developmental rates of cleavage, morula and blastocysts during breeding season than non-breeding season but there was no significant difference between using either right or left oviductal extraction on the previous developmental rates.

Keywords: Uterus, She-camel, Arterial supply, Histology, In-vitro production of camel embryo.

INTRODUCTION

Despite the importance of the dromedary camels as high milk and meat productivity but the reproductive performance is still limited, moreover using of assisted reproductive technologies as IVF, gamete penetration, superovulation and somatic cell nuclear transfer need more studies (Fathi et al., 2014. Wani et al., 2010).

Since many years, the scientists were attempted to clarify the left uterine horn pregnancy in she-camel. Most of them aligned with the fact that, the left uterine horn had much nourishment either through arterial blood supply or via the uterine glands which may gave much supply to the she-camel fetus. Although, the ovulation process in the she-camel occurred from both ovaries with the same frequency but the pregnancy only occurred in the left uterine horn by a rate of 99.5% and none in the uterine body while 0.48% in the right uterine horn of the 414 examined specimens (El-Wishy, 1988; EI-Swafy, 1971; Ghoneim, 1985; Abd-Elnaeim, 1998; El-Gindy, 1982).

Many theories were studied to explain the very high percentage pregnancy in the left uterine horn as: embryo migration by (Ghoneim, 1985)

and death of the embryos developed in the right horn as it was weakly developed (Arthur et al., 1986).

Descriptive information about the utero-ovarian vasculature in she-camel has been described (El-Gindy, 1982). However, the detailed arterial distribution of the uterine vessels has been ignored for years, and currently no adequate reports were available about their distribution. These information gaps add to the effort of studies on reproduction and reproductive organs, and lead to misunderstanding of the results of different experiments (Shireen 2005).

The uterine wall can divide functionally into the endometrium and myometrium (Atkinson et al., 1984; Wiley et al., 1987). Uterine histological structure in camel are similar to those of other domestic species with few distinct uniqueness (Tibary and Anouassi, 2000). The lamina propria of camel endometrium consisted mainly of stromal cells of irregular shape, and collagen fibers, which present in significant amounts than that in other species (Fetaih et al., 1992). The endometrium supported by a broad, highly cellular connective tissue stroma with relatively few simple tubular glands that open in the surface of the epithelium. A relatively thick layer of myometrium surrounded the endometrial tissue (Tibary and Anouassi, 2000; Srikandakumar et al., 2003).

According to the above mentioned reasons, we aimed to give a recent image on the arterial blood supply, histology on the uterus of she-camel as well as to study the effect of adding oviductal extracts on the culture medium on the developmental competence on dromedary she-camel oocytes.

MATERIALS AND METHODS

Twenty non-pregnant uteri of the clinically healthy one-humped she-camel obtained shortly after slaughtering from Cairo and Giza slaughterhouses. Ten out of twenty collected during the breeding season, winter period and the other ten collected during the non-breeding season, summer period. Age of the she-camel ranged from 8 to 11 years old.

For the anatomical study:

Major blood vessels wall thickness were measured using Vernier Caliber in all fresh specimens. All measurements were tabulated and the data obtained were statistically analyzed using the SPSS statistical software (version 22.0, SPSS, USA).

To study the arterial blood supply of the uterus, six specimens were cannulated through the ovarian and the utero-vaginal arteries and flushed thoroughly with normal saline then injected with 60% gum milk latex emulsion colored red using ROTRING ink. Then the specimens were immersed in solution of formalin 10% and 1% glycerin for 4 days before manual dissection (Hildebrand, 1968). The photographs were taken using NIKON digital camera 12 megapixels.

For the histological study:

Six uteri used for this study and the right and left she-camel uterine horn were fixed in 10% neutral buffered formalin for 72 hrs. Samples were trimmed and processed by dehydration in serial grades of ethanol, cleared in Xylene, synthetic wax infiltration and embedding into Paraplast tissue embedding media. 4µn thick tissue sections were cut by rotatory microtome and fixed to glass slides. The sections were stained with: 1-Harris Hematoxylin and Eosin as a general examination staining method for histological evaluation. 2-Masson's Trichrome stain for demonstration of collagen fibers. 3- PAS technique for demonstration of neutral mucopolysaccharides. As outlined by (Bancroft and Layton, 2013).

Morphometric measurements:

mean percent of mucosal glandular tissue were selected and obtained from 6 non overlapping microscopic fields as well as mean endometrial (mucosal) thickening using full HD microscopic camera operated by leica application module for tissue sections analysis (Leica biosystems- Germany). The data obtained were statistically analyzed using the SPSS statistical software (version 22.0, SPSS, USA).

For the in-vitro production of camel embryo:

In vitro maturation, fertilization and culture of dromedary camel oocytes were done as previously described (Fathi et al., 2014, Fathi et al., 2018). In brief, the recovered cumulus oocyte complex (COCs) were matured using TCM-199 supplemented with (10%FCS, 10 µg FSH, 50 µg/ ml sodium pyruvate and 50 µg/ ml gentamycin), for 30 hours in 5% Co2 in high humidity air. Matured oocytes were inseminated using camel epididymal spermatozoa that flushed from cauda epididymis and capacitated using 5 Mm caffeine as previously described by (Fathi et al., 2018), matured oocytes were incubated with the capacitated spermatozoa for 18 hours. The harvested zygotes were cultured in potassium

simplex optimized medium supplemented with either right or left oviductal fluid extract during either breeding or non-breeding seasons**.**

RESULTS

A- Anatomical findings:

The morphological anatomy of the she-camel uterus shown in (Fig. 1). The she-camel uterus classified as a bipartite uterus and the left uterine horn was markedly larger than the right one.

The uterus of the one- humped she–camel, were supplied by the uterine and ovarian arteries. In all specimens examined, the main arteries in the left side were longer, more flexuous, of greater diameter and thicker than those of right ones in the same animal. Anatomically, in both breeding and non-breeding season, there were no difference between the pattern of distribution of the arteries suppling the ovary and the uterus except that in the breeding season, the arteries diameter became much higher and more flexuous.

1- Ovarian artery (Fig 2&4/1):

The right ovarian artery arises from ventral aspect of the abdominal aorta cranial to the external iliac artery on a level with the 5th lumber vertebra. on the left, it emanates from the caudal mesenteric artery soon after it originates from the abdominal aorta and a little caudal to the right one. Each proceed in a flexuous course cranio-ventrally and they continue up to 5 cm caudal to the ovary, where each divides into ovarian, tubal and uterine branches.

2- Ovarian branch of the ovarian artery (Fig 2&4/2):

The ovarian branch proceeds cranio-laterally being enclosed in the meso-ovarian ligament. Before reaching the attached border of the ovary it divides into 3-4 twigs that enter the ovary.

3- Tubal branch of the ovarian artery (Fig 2&4/3):

The tubal branch passes cranially in the mesosalpinx forming a highly convoluted course. At the junction of the isthmus with the uterine horn, the tubal branch detaches 3-5 fine twigs that anastomose with the uterine branch. In only two case, the right tubal branch divides into two branches on a level with the ovary. one branch passes medially to anastomose with the uterine branch while the other proceeds to the uterine tube.

4- Uterine branch of the ovarian artery (Fig 2&4/4):

From the point of size, the uterine branch constitutes the largest of the three terminal branches. it passes cranially in a flexuous course where it reaches the middle of the caudal border of the uterine horn. Here the artery turns medially and terminates into 3-5 small twigs which anastomose with the corresponding ones from the lateral branch of the ventral uterine artery. During its course, it gives off 4-6 twigs from its lateral aspect. they anastomose with branches from the tubal one in addition to 6-10 small twigs from its concavity to supply the cranial half of the uterine horn.

5- Utero-vaginal artery (Fig 2, 3&4/5):

It was a large sizes trunk common for the uterine and vaginal arteries. It originates from the umbilical artery at level of the 6th lumber vertebra.

6- Vaginal artery (Fig 2&4/6):

The vaginal artery arises in a common trunk with the uterine artery from the umbilical on a level with the 6th and 7th lumbar vertebrae. The artery bifurcates into a smaller cranial and a larger caudal one. The former extends cranially on the lateral aspect of the cervix where it gives off 4-6 twigs which are distributed on the cervix and anastomose with both the ventral and dorsal branches of the uterine artery. The later terminates at the cranial part of the vagina.

7- Uterine artery (Fig 2, 3&4/7):

The uterine artery is a very strong vessel measuring about 30-40 cm in the non-gravid organ. It arises in common with the vaginal artery from the umbilical artery on a level with in between the 6th and 7th lumbar vertebrae. In two specimens, the uterine artery originated from the internal iliac artery on a level of the 6th lumbar vertebra. The uterine artery descends caudo-ventrally in the caudal third of the broad ligament of the uterus forming an acute angle that carries the artery in a cranial direction on the lateral aspect of the cervix. At the middle third of the latter, each artery divides into a strong more flexuous ventral branch and a weaker less flexuous dorsal one. The former, courses cranially within the texture of the ventral aspect of the body of the uterus in a coiled manner till it reaches its middle, where it bifurcates into two primary branches, lateral and medial. Such division occurs at the junction of the body with the cervix of the uterus on the right side.

8- Dorsal branch of the uterine artery (Fig 2, 3&4/8):

The dorsal branch of either side arises at the middle third of the cervix from the parent trunk. It proceeds cranially within the texture of the dorsal aspect of the body of the uterus till the intercornual area where it divides into three branches which re-divides again and anastomose

with similar ones from the ventral branch on the cranial border of the corresponding uterine artery. During its course, it gives off 8-10 secondary branches (Fig 3/ 16) from both sides which pass transversely on the dorsal aspect of the uterine horn in and anastomose with twigs from the ventral branch of the uterine artery. Such anastomoses occur at the cranial border of the uterine horn.

9- Ventral branch of the uterine artery (Fig 2, 3&4/9):

The ventral branch ran cranially in a highly flexuous course through the ventro-caudal aspect of the uterus. At about the middle of the uterine body, it bifurcated into two primary branches: lateral and medial branches.

10- Lateral branch (Fig 2&4/10):

proceeds cranio-laterally towards the junction of the body with the uterine horn to form an arch which continues on the caudal border of the uterine horn and terminates into two branches which in turns re-divide again to give secondary branches that ramify on the caudal half of the uterine horn and anastomose with uterine branches of the ovarian artery.

11- Medial branch (Fig 2&4/11):

Passes cranio-medially into the intercornual area where it divides into three branches. Each one divides into secondary branches of the opposite side. It detaches 8-10 branches from both sides which supply the middle part of the body of the uterus and anastomose with similar ones of the lateral branch.

12- Secondary (Fig 4/12) and 13- Tertiary branches (Fig 4/13):

The medial branch divided into 4 - 5 secondary branches then re-divided into a number of tertiary branches which anastomosed with similar ones of the opposite side. this network aimed to supply the intercornual area of the uterus.

14- Cranio-lateral branch (Fig 2&3/14) and 15- Craino-medial branch (Fig 3/15):

The lateral branch divides into 2 branches, the cranio-lateral and the craino-medial branches. The two branches gave 10 -12 secondary branches on the caudal half of the uterine horn and on the intercornual region which anastomosed with similar ones from the dorsal branch of the uterine artery on the dorsal aspect of the uterine horn.

The arterial wall thickness of the major blood vessels suppling the uterus were measured, statically analyzed, Table (1) and showed that the left side blood vessels were mostly thicker than those of right ones in the same animal at the breeding season except in three specimens, the right dorsal branch of the right uterine artery recorded much higher thickness than the left one in the same animal but the distribution of secondary and tertiary branches on the left side always much higher than in the right side.

Histological findings:

Histologically, the Uterine Horn in breeding season composed of endometrium which lined with ciliated columnar epithelium with underlying lamina propria (stroma) composed of highly cellular and vascularized connective tissue (Plate 1/A). In addition, the endometrium showed irregularly raised longitudinal uterine folds and ridges (Plate 1/A&B) surrounded by a thick layer of myometrial tissue (Plate 1/C).

The endometrial glands were localized in two different regions; adjacent to luminal region of endometrium close to the luminal epithelium and in the region of endometrium close to smooth muscles layer of the myometrium (Plate 1/A&B).

The endometrial lining consisted of a single layer of columnar epithelial cells supported by a broad highly cellular connective tissue with simple tubular glands (Plate 1/D).

Structurally there was no significant difference between left and right uterine horn. In non-breeding uterine horn had the same histological structure with remarkable less number of endometrial gland in lamina propria (Plate 1/E).

The uterine gland and endometrial epithelium react similarly faint purple to PAS in both left and right uterine horn (Plate 1/F&G).

Statistically, by image analysis the endometrial thickness of the breeding right horn was measure 2700 µm while left uterine horn was 2200 µm, in the same regards the difference in the quantification of endometrial glands were represent 25% and 19 % of the mucosa in right and left horn respectively. Meanwhile the non-breeding left and right uterine horn showed no significant difference in mucosal thickness and the glandular tissue percentage between both.

C- In vitro production of camel embryo:

Table (2) The percentages of cleavage, morula and blastocysts produced following IVC of fertilized oocytes using either right or left oviductal fluid extract in breeding and non-breeding seasons.

Table (1) The arterial wall thickness (in micrometer) mean and standard error of the major arteries suppling the uterus in breeding and non-breeding season of adult She-Camel (8 – 11 years).

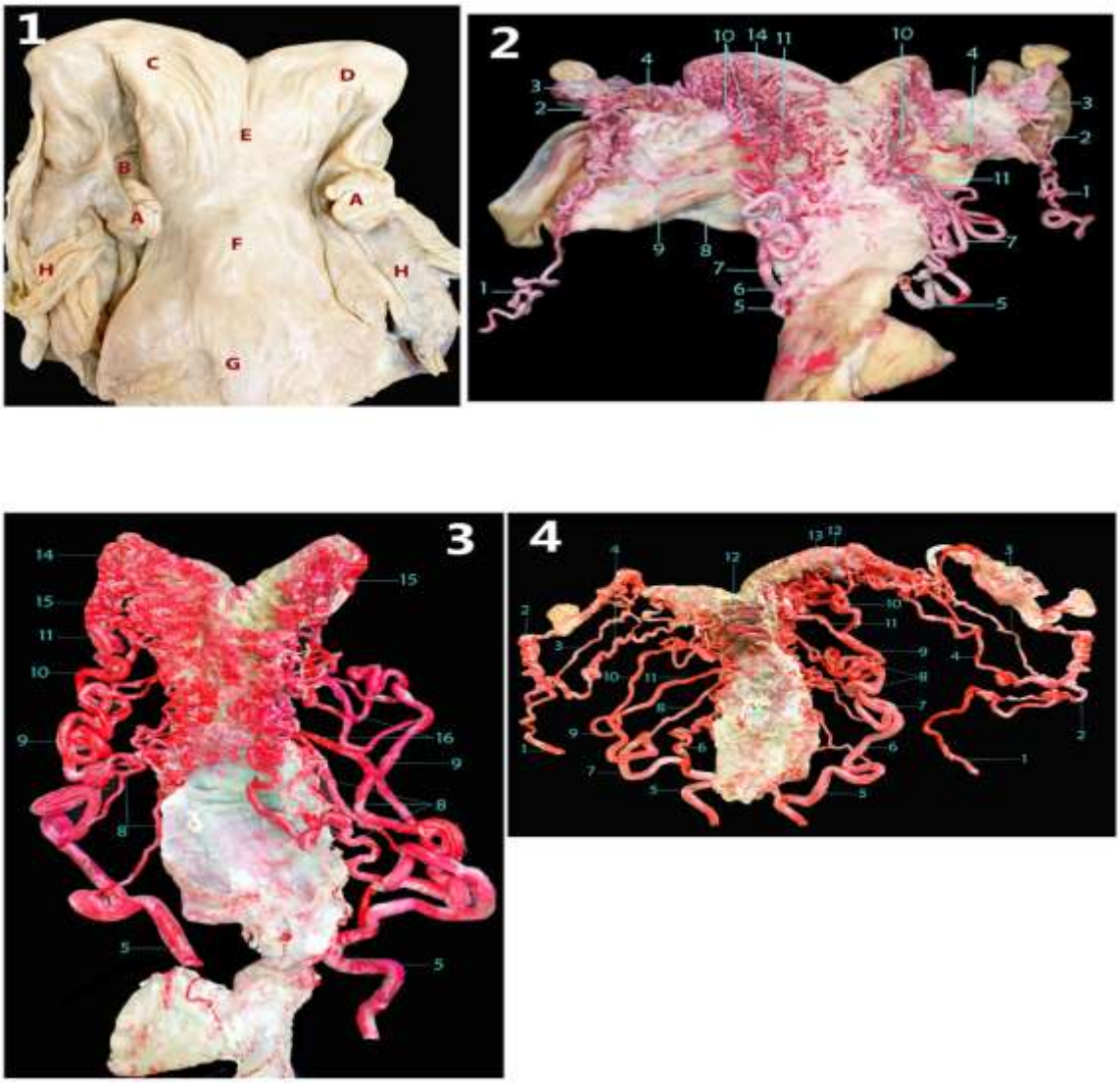
Artery	Non-breeding		Breeding	
	LEFT (µm) (mean±SEM)	RIGHT(µm) (mean±SEM)	LEFT(µm) (mean±SEM)	RIGHT(µm) (mean±SEM)
1-Uterine branch of the ovarian artery	(117.5±2.20)^{a}	(112.5±2.82)^{a}	(227±4.55)^{b}	(217±3.35)^{b}
2-Uterine artery	(289.5±3.90)^{b}	(281.5±1.55)^{b}	(634.5±5.65)^{d}	(629.0±4.50)^{d}
3-Ventral uterine artery	(257.5±3.35)^{b}	(255.0±2.25)^{b}	(641.0±5.30)^{d}	(628.0±4.40)^{d}
4-Lateral branch of the ventral uterine artery	(207.0±1.15)^{b}	(203.0±1.20)^{b}	(430.5±2.25)^{c}	(418.0±3.35)^{c}
5-Medial branch of the ventral uterine artery	(236.0±0.80)^{b}	(232.0±0.95)^{b}	(558.5±5.56)^{d}	(570.0±5.54)^{d}
6-Dorsal branch of the uterine artery	(229.0±1.60)^{b}	(226.0±1.25)^{b}	(594.0±6.54)^{d}	(600.0±4.80)^{d}

Different superscripts in the same column are significantly different at P<0.05.

Table (2) The percentages of cleavage, morula and blastocysts produced following IVC of fertilized oocytes using either right or left oviductal fluid extract in breeding and non-breeding seasons.

Fertilized oocytes (cultured in IVC medium supplemented with)	N	Cleavage (2-16 cell stage) N (mean% ±sem)	Morula N (mean% ±sem)	Blastocyst N (mean% ±sem)
Left oviductal fluid extract (breeding season)	54	18 (33.3±1.20)^{a}	10 (18.5±0.85)^{a}	5 (9.2±0.54)^{a}
Right oviductal fluid extract (breeding season)	62	19 (30.6±1.11)^{a}	11 (17.7±0.94)^{a}	5 (8.1±0.67)^{a}
Left oviductal fluid extract (non-breeding season)	66	12 (18.1±1.65)^{b}	5 (7.5±1.31)^{b}	2 (3.1±0.43)^{b}
Right oviductal fluid extract (non-breeding season)	59	9 (15.2±1.22)^{b}	4 (6.7±1.05)^{b}	2 (3.3±0.52)^{b}

Different superscripts in the same column are significantly different at P<0.05.

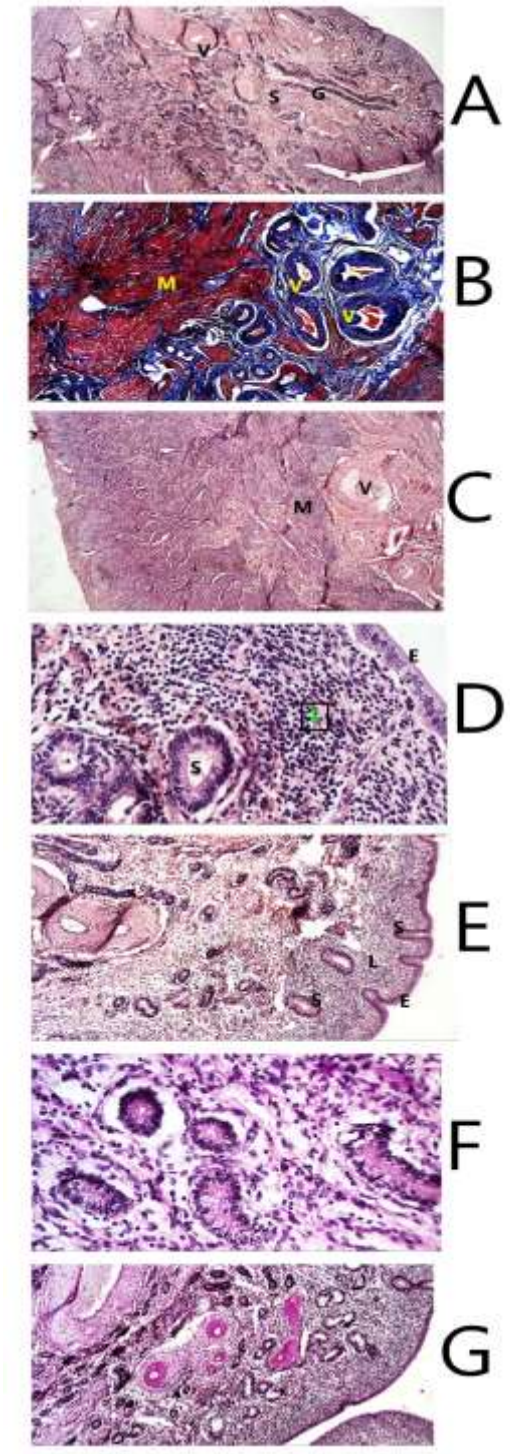


A- Ovary	B- Uterine tube
C- Left uterine horn	D- Right uterine horn
E- Body of the uterus	F- Cervix
G- Vagina	H- Broad ligament
1- Ovarian artery	2- Ovarian branch
3- Tubal branch	4- Uterine branch
5- Utero-vaginal artery	6- Vaginal artery
7- Uterine artery	8- Dorsal uterine artery
9- Ventral uterine artery	10- Lateral branch
11- Medial branch	12- Secondary branch
13- Tertiary branch	14- Craino-lateral branch
15- Cranio-medial branch	16- Secondary branch of dorsal uterine artery

Figure. (1): A photograph showing the gross anatomy of a non-pregnant reproductive tract of she-camel.
Figure. (2): A photograph showing the arterial distribution of the uterus and ovaries during the breeding season in non-pregnant she-camel (10 years). Dorsal view.
Figure. (3): A photograph showing the arterial distribution of the uterus during the breeding season in non-

pregnant she-camel (11 years). Dorsal view.

Figure. (4): A photograph showing the arterial distribution of the uterus and ovaries during the breeding season in non-pregnant she-camel (11 years). Ventral view.

	**A: Histological section of left uterine horn of she camel in breeding season showing endometrial gland (G); endometrial stroma (S) and vascular layer (V). H& E X400.** **B: Histological section of left uterine horn of she camel myometrium (M) and Vascular layer (V). Mason's Trichrome X40.** **C: Histological section of left uterine horn of she camel showing thick Myometrium layer (M), External layer and vascular layer (V). H& E X40** **D: Histological section of left uterine horn of she camel in breeding season showing Simple columnar epithelium (E); simple tubular gland (S) and highly cellular lamina propria (L). H& E X100.** **E: Histological section of non-breeding uterine horn of she camel showing Simple columnar epithelium (E); simple tubular gland (S) and highly cellular lamina propria (L) and vascular layer (V). H& E X40.** **F: Histological section in left uterine horn of she camel in breeding season showing faint PAS reactivity in gland and surface epithelium PAS X 40.** **G: Histological section in right uterine horn of she camel in breeding season showing faint PAS reactivity in gland and surface epithelium PAS X 40.**

DISCUSSION

The present study classified the she-camel uterus as a bipartite uterus in accordance with (Srikandakumar et al., 2003 and Eman, 2006) that was not in agreement with the earlier classification by (Arthur et al.,1986) who described a camel's uterus as bicornuate. This classification supported the fact that the live twin births did not happened in the uterine body of the she-camel.

The present study demonstrated that, the mode of division of the ovarian artery in the she-camel into ovarian, tubal, and uterine branches resembled to that given in the goat, (Shireen 2005); (Volimerhaus 1964), in the cow; (Mobarak,1967) and (Badawi & Abd El-Raouf, 1970) in the buffalo and in the camel, (Ghazi, 1981 and El-Gindy et al, 1982).

In she-camel, in contrast to other ruminants (Ghazi, 1981 and El-Gindy et al, 1982), there was no middle uterine artery. As the present study revealed, it had been found that, both the vaginal and the uterine arteries arose by a common trunk from the umbilical artery. A finding which recorded also in the cow (Volimerhaus 1964), in the bitch (Miller, Christensen and Evans, 1974), in the goat (Shireen 2005) and in the she-camel (El-Gindy et a.l, 1982 and Srikandakumar et al., 2003). On the other hand, Chauveau (1891), Nickel et al., (1960), Schummer and Seiferie (1960), Getty (1975) in the cow and Mobarak (1968) in the buffalo, stated that the uterine artery originates from the internal iliac artery a result which is not seen in the investigated material.

The present investigation showed that, in the she –camel, the uterine artery divided into dorsal and ventral primary branches and each re-divided into secondary branches which anastomoses with the uterine branch of the ovarian artery. In cow, Hilliger (1958), in goat, Shireen (2005) and in buffalo Mobarak (1968) mentioned that such artery give rise to primary and secondary branches in a fan shape arch form manner which does not occur in camel.

In the literature it has been found that, in the cow, Hilliger (1958) described three different modes of branching of the uterine artery. They were the fan-like, arcade and horn-parallel types. These patterns were also observed in the buffalo (Badawi & Abd El-Raouf, 1970). The horn-parallel type as a mode of branching was the most common type in the present study. In addition to the incidence of both individual secondary uterine and anastomotic branches between the arteries of the uterine horns.

In data obtained showed that, in the breeding season, the arterial wall thickness of the major blood vessels suppling the uterus on the left side were thicker than those of right ones in the same animal, this was in accordance with (Adel et al, 1988) in the she-camel. While the author added that in three specimens, the right dorsal branch of the right uterine artery recorded much higher thickness than the left one in the same animal that was not mentioned by the data recorded by (Adel et al., 1988). Despite the distribution of secondary and tertiary branches on the left side always much higher than in the right side, in accordance with (El-Gindy et al., 1982) in the Camel.

The camel is seasonal polyestrous with induced type of ovulation (Sghiri and Driancourt, 1999). In the present sturdy, the histological structure of the uterus of she –camel was contained three distinct layers similar to other mammalian uteri, but the nature of the endometrial layer was differed from the adult sheep and cow endometrium, which consisting of both aglandular caruncular areas and glandular inter-caruncular areas (Atkinson et al., 1984; Wiley et al., 1987). The endometrial gland was simple, branched tubular glands; this finding was similar to that found in the other animals (Wiley et al., 1987; Taylar et al., 2000; Gray et al., 2001).

In she -camel ages 8-11 years all the morphometric measurements of the layers of wall of all uterine parts at winter were higher than those at summer and there were significant variations at thickness of the endometrium of the right and left horns, this result may be due to nutritional status as described by Tibary and Anouassi, (2000) which lead to the maximum ovarian activity during the winter , these cause high level of oestradiol during the peak breeding seasons (Agarwal and Khann, 1990 ; Cristofori and Quaranla , 1990; Sghiri and Driancourt 1999) and low level of estrogen in the non- breeding seasons (May – November) (Elias et al ., 1984) .

Olivera et al., (2003) described that the differences in the glandular density and staining of the epithelial and glandular cells of endometrium in both follicular and luteal phase was possibly due to the hormonal balance of each specific phase and organelles density, that might be a point of study to the hormonal receptor balance and other factors might include in difference in glandular tissue intensity in endometrium of right horn in breeding season and left horn.

The results of one study revealed that, a significant increase in developmental rates of cleavage, morula and blastocysts during breeding season than non-breeding season but there was

no significant difference between using either right or left oviductal extraction on the previous developmental rates.

Nearly higher than our findings were found by (Fathi et al., 2017) they were found that the cleavage, morula and blastocyst rates were (45%.30% and 19%) this may be attributed to using BCB stain for selection of more competent oocytes

CONCLUSION

In conclusion, the arterial distribution of the uterus in she-camel showed higher diameter thickness and higher distribution on the left side of the uterus than the right side. The endometrial thickness of the breeding right horn was higher than the left one. Meanwhile the non-breeding left and right uterine horn showed no significant difference in mucosal thickness and the glandular tissue percentage. There was no significant difference between using either right or left oviductal fluid extract during IVC on the developmental rates of camel embryos, further studies are needed upon maternal recognition factors to emphasis the left horn pregnancy phenomenon.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Special acknowledgment for our colleagues from Faculty of Veterinary Medicine, Cairo University for their valuable support to complete this study; Dr. Mohamed Abdelrazik, Lecturer of Cytology and Histology, Dr. Amr El-Karmoty, Associate Professor of Anatomy and Embryology, and Prof. Dr. Yasser Wally, Professor and Head of Anatomy & Embryology Department.

AUTHOR CONTRIBUTIONS

Design of research: MF and AT; Methodology: AT, SM, MA and MF. In vitro study: MF. AT, MA and MF wrote the manuscript and data collection. Data analysis: SM, AT, MA and MF. experiments and reviewed the manuscript. All authors read and approved the final version.

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