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Histological and ultrastructural studies of female reproductive vasculature of one humped camel in relation to possible thermoregulation and ovarian hormones

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Abstract

The study was designed to explore anatomical and histological vasculature changes in the female genital system of camel and serological aspects that might be responsible to maintain thermoregulation. Twenty-four adult female camels were sampled during breeding (November-April) and non-breeding (May-October) season. Blood was collected for estrogen, progesterone and cortisol level estimation. Genital organs were sampled and described after slaughtering. Samples were taken from the ovarian artery (OA), vein (OV) and arterio-venous complex (AVC), for light and scanning electron microscopy. Sections were stained with Hematoxylin and Eosin. (H&E), Masson's trichrome, Weigert's elastic and toluidine blue. Temperature and relative humidity were used to calculate stress indicator. Stress indicator was higher in non-breeding season (NBS). Anatomical and histological vasculature (OA, OV, AVC) dynamics were significantly higher in breeding season (BS) especially diameter of left OA. Parameters of OA were positively associated with estrogen level. Collagen, elastic, smooth muscles and mast cells were recorded least in BS compared to NBS. Unique venous structure, intra-mural venules (IMV), was discovered in tunica intima of OA, seen positively and negatively associated with estrogen and cortisol level in BS, respectively. Scanned electron-micrograph exhibited penetration and wrapping of OA by small thinned-walled venules that may form IMV. The AVC was too tightly packed to differentiate due to the collapse of the wall. Hormonal, seasonal, stress indicator and vascular dynamic of female genital system are interlinked and IMV in association with OA and OV may be proposed as the site of counter-current exchange in female reproductive system of the camel.

Introduction

Thermoregulation is a control mechanism of the central and peripheral nervous system integrated at a preoptic area of the anterior hypothalamus (PO/AH) which is the primary site of the nervous system responsible for coordination and control of heat responses.[1] The PO/AH senses any change in external and internal temperature and try to maintain core body temperature at a fixed level by down or up-regulating heat loss and conservation via various mechanisms like vasodilation, sweating, vasoconstrictions and shivering.[1,2]

It is not only high temperature that affects animal health, but its combination with relative humidity (RH) is also of prime importance.[3] It means that high temperature with low RH may not be as harmful to animal health as moderately high temperature with high RH and vice versa. Therefore, a thermoneutral zone i.e., 60 - 72 thermal-humidity index (THI) for ruminants is defined for the better productive and reproductive performance of animals.[4]

A higher value of THI than the thermal neutral zone of the mammals (60 - 72 THI for ruminants) is a stress that likely to cause death due to interruption in the protein structure, membrane stability, fluidity, fluid and electrolyte loss.[5] Failure in reproductive thermo-regulation especially in high producing animals results in inappropriate oocyte capacity for fertilization and

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optimum development of an embryo.[5,6] The livestock has been the main source of income in developing countries including Pakistan.[7-9] Among livestock, Camel is considered a preferred source of food than other dairy animals [10] because it can withstand extreme weather conditions like during summer (47.00 - 50.00 ˚C) and winter (10.00 - 20.00 ˚C).[11] The reproductive performance of camels under natural ecology is very poor due to short breeding season, prolonged calving interval and early embryonic mortality.[12] Mostly these problems are associated with seasonal trend of breeding which remained still unclear[13] and reproductive thermo-regulation issues. The reproductive vasculature of cow, sheep and swine contain a vascular complex in which small branches of utero-ovarian arteries wind around uterine veins extensively for effectively exchange heat to protect the reproductive system from heat stress. This arterio-venous complex maintains the reproductive thermoregulation and hormonal exchange. The exact site of heat exchange, structure and seasonal variations in arterio-venous complex and hormonal control of this structure in female camels are still unexplored. Therefore, this study was undertaken to describe the anatomical and histological changes in the walls of ovarian and uterine blood vessels that maintain the thermoregulation in the female genital system of camel (*Camelus dromedaries*).

Materials and Methods

This study was conducted with the approval and permission of the Animal Ethics Committee of the University of Agriculture-Faisalabad (Approval Number: 203402-401, dated: 3.01.2019). The collection of samples of the reproductive system of the female camel was done after a routine slaughtering procedure. No animal was harmed otherwise. In the present study, Twenty-four adult female camels were sampled that were presented for being slaughtered at a local abattoir in Faisalabad-Pakistan during extreme reproductive inactivity (June-August), the start of reproductive activity (September-October), the peak of reproductive activity (November-February) and declining of reproductive activity (March-April). Former two groups were combined as non-breeding (May-October) and the latter were combined in breeding (November-April) seasons. Six female camels, above five years of age, were sampled during each phase of the reproductive year of camel (n = 6). Age of camels was determined by dentition as described by Rabagliati.[14] Body temperature, respiration rate and heartbeat were recorded to verify the health status of each camel.

Blood collection. Blood (10.00 mL) was collected in vacutainer without anticoagulant from the external jugular vein of each animal just before slaughter. Blood was thoroughly mixed by inverting the vacutainer several times and let it settle for 30 minutes. The vacutainers were then centrifuged at 500 g for 10 minutes to separate the serum. Serum was aspirated, labeled and stored at – 20.00 ˚C in Eppendorf tubes until further processing.

Collection of female genital system. The female genital system of one humped camel was collected immediately after slaughtering. The entire genital system was examined and photographed immediately after slaughtering. Ovarian weight was determined to the nearest 0.01 g. Ovarian circumference, width and oviductal diameter were determined to the nearest 0.10 mm.

Tissue isolation and processing. The ovarian artery (OA), ovarian vein (OV) and arterio-venous complex (AVC) of vessels were separated and fixed in 10.00% neutral buffered formalin solution before processing by standard paraffin histological techniques.[15] Sections of 5.00 - 7.00 µm thickness were cut and mounted on glass slides and stained with Hematoxylin and Eosin (H&E). The sections were also stained with Masson's trichrome stain to visualize collagen contents and Weigert's elastic stain to visualize elastic contents. Sections of 6.00 µm thickness were also stained with toluidine blue (1.00% aqueous solution, pH 1.00; and 5 min). The stained slides of OA and OV were analyzed to measure overall thickness of different tunics of vessels, elastic and collagen fiber contents in all tunica. The number of mast cells per unit area (mm^2) was counted by the method used by the Karaca *et al*.[16] All measurements were performed with computer software Image J analysis system.

Serum biochemistry. Corticosteroids and female sex hormones (estrogen and progesterone) were determined by radioimmunoassay (RIA) using commercially available kits (Randox Laboratories, Crumlin, UK).

Thermal-humidity index (THI). For THI calculation, temperature and relative humidity data during sampling were collected from the Metrological Department, Faculty of Agriculture, University of Agriculture-Faisalabad, Pakistan. Mean THI was calculated for all four reproductive phases as well as their combined groups. THI calculated by the given equation:

$$THI = (1.80 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.80 \times T - 26)$$

where, *T* represent temperature (˚C) and *RH* is relative humidity (%).[4]

Scanning electron microscopy. Perfusion was done with 4.00% paraformaldehyde into the blood vessel segments. This excised tissue was immersed in 2.00% paraformaldehyde and 3.00% glutaraldehyde in DMEM (Life Technologies Corp, Grand Island, USA) buffered with 10.00 mM HEPES (Hopax Chemicals, Kaohsiung, Taiwan) with pH 7.40 for 1 hr. This room-temperature fixation was followed by a 30-min incubation on ice with 0.10% OSO4 (Sigma Aldrich, St. Louis, USA) in 0.10 M phosphate buffer, pH 6.00. The tissue was then washed in three changes of deionized water, then dehydrated in a graded series of ethanol. After dehydration, the vessel segments were

critical-point dried (Sandri-790; Tousimis Research Corporation, Rockville, USA) and sputter coated. Blood vessels were examined on an International Scientific Instrument scanning electron microscope (S-2380N, Hitachi, Tokyo, Japan) operated at 9.00 kV.

Statistical analysis. One-way analysis of variance (ANOVA) under complete randomized design was applied to means of parameters calculated by statistics software R (version 3.4.1; R Foundation, Vienna, Austria). Turkey's Honestly Significant (THS) test was used as post ANOVA test at 5.00% level of significant. To correlate different parameters, the coefficient of correlation (R) was also calculated.

Results

Female reproductive vasculature. Gross anatomical examination of reproductive vasculature revealed that OA, originates directly from the abdominal aorta and gives off three main branches i.e., anterior uterine, ovarian and oviductal arteries that supplies blood to the corresponding uterine horn, ovary and oviduct, respectively. The venous blood from aforementioned parts is collected via branches of OV satellite to adjacent arteries. The utero-ovarian branch of utero-ovarian vein was wrapped with the extensive twisting pattern of the ovarian branch of ovarian artery which formed a special vascular complex called AVC. The uterine body was vascularized by the uterine artery and no middle uterine artery was observed in the reproductive system contrary to the bovines.

Gross parameters. Ovarian dynamics are greatly influenced by season as shown in Table 1. Ovarian width and circumference were significantly ($p < 0.05$) higher in breeding season (BS) compared to non-breeding season (NBS). However, these parameters of left and right sides within season remained non-significant ($p > 0.05$). Significantly ($p < 0.05$) high weight was recorded on left side ovary in BS (11.82 ± 1.10 g). The season did not influence length of oviduct ($p > 0.05$) but over-all wider diameter (5.72 ± 0.32 mm) was recorded in BS compared to NBS. Left uterine horn was found almost twice longer than the right regardless of season while a significant ($p < 0.05$) increase in the length of left uterine horn was seen in BS as compared to NBS. The change in the right uterine horn was non-significant ($p > 0.05$) during BS and NBS.

Histological parameters. Statistical analysis of microscopic parameters of left and right uterine artery are given in the Table 1. Left OA, in BS, showed significantly ($p < 0.05$) wider diameter (250.01 ± 13.20 mm) than that of right side as well measured in NBS. Tunica media of left OA was significantly ($p < 0.05$) thicker (240.40 ± 13.10 µm) in BS as compared to all groups regardless of side while seasonal effect show no statistical significance on tunica intima of the artery. The main arterial and venous blood to and away from the ovary/uterine wall were regular muscular vessels with relatively thick wall (Figs. 1A and 1B). The thickness of the tunica media though started to decrease as the vessels divides close to the arteriovenous plexus of the ovary (Figs. 1C and 1D). At certain points, the wall of the ovarian arterial branch showed a decrease in wall thickness and at the same time large number of venous branches surrounded artery (Figs. 1E and 1F). At this specific region, thin-walled veins showed large number of valves within the lumen (Fig. 1I). The close relationship between the arterial and the venous branches was clear as the vessels approached the ovary and the horn of the uterus. Plenty of dilated venous spaces with regular and irregular lumen were observed (Fig. 1J). The elastic and collagen fibers and smooth muscle contents (39.60 ± 2.70, 21.60 ± 2.10 and 33.20 ± 1.10%, respectively) were recorded higher ($p < 0.05$) in the right OA during NBS compared to BS. By comparing OA and OV supplying the uterus regardless of side and season, fibers (elastic and collagen) and smooth muscle contents were higher significantly ($p < 0.05$) in vein than artery except for tunica media which showed otherwise pattern (Fig. 2A). The area of small-sized veins presents in tunica intima of the ovarian artery, intra-mural venules, were observed significantly ($p < 0.05$) more in left side artery in all season (Table 1, Fig. 1G and Fig. 2B). The mast cell in the AVC complex was counted significantly more ($p < 0.05$) in NBS as compared to BS (Table 1and Fig. 1H).

Electron microscopy. Scanning electron microscopy of the different sections of the ovarian artery in BS and NBS was done before entering into the AVC. These micrographs showed larger number of IMV during BS as compared to NBS. Micrograph of outer layer of the OA presented the extensive wrapping of the artery by the small-sized venules and penetration into the walls of the artery. Tunica adventitia contained numerous fibrous lamella and an anastomosing pattern of small-sized venules. In AVC, sharing of different tunics of artery and veins was observed which was difficult to differentiate the boundary of vessels (Fig. 3).

Hormonal parameters. Quantitative stress indicator, THI, was calculated highest (90.00 to 95.00) in the months of peak NBS-extreme reproductive inactivity phase of the year as shown in the Figure 4A. In peak reproductive activity THI was found between 60.00 and 70.00. Serum estrogen level of female camel exhibited increasing trend during different phases of BS (Table 2, Fig. 4B). Significantly lowest (21.33 ± 2.66 pg mL^{-1} level of estrogen was witnessed in peak NBS or extreme reproductive inactivity phase (June-August) and highest (63.40 ± 4.82 pg mL^{-1}) in the peak of reproductive activity of BS (November-February). Estrogen level during different phases of BS was observed negatively affected by THI values in different phases while the cortisol curve followed the direct trend with THI (Table 2, Fig. 4B).

Table 1. Morphometrical values of different parts of female camel reproductive system, histological aspects of uterine artery and serum hormonal level of different hormone (estrogen, progesterone and cortisol) during breeding season (BS: November-April) and non-breeding (NBS: May-October).

Parameters	Breeding season		Non-breeding season		Overall Means	
	Left	Right	Left	Right	BS	NBS
Morphometry of different parts of female reproductive system of camel						
Ovarian width (cm)	2.84 ± 0.25^{a}	2.66 ± 0.27^{a}	2.38 ± 0.17^{b}	2.09 ± 0.18^{bc}	2.75 ± 3.20*	2.23 ± 2.01
Ovarian circumference (cm)	7.72 ± 0.42^{a}	7.02 ± 0.24^{a}	6.22 ± 0.14^{b}	5.81 ± 0.14^{b}	7.37 ± 0.32*	6.01 ± 0.15
Ovarian weight (g)	11.82 ± 1.10^{a}	10.15 ± 0.58^{b}	6.95 ± 0.51^{c}	6.20 ± 0.96^{c}	11.00 ± 0.98*	6.55 ± 0.72
Diameter of oviduct (mm)	5.62 ± 0.24^{a}	5.82 ± 0.26^{a}	4.62 ± 0.46^{b}	4.63 ± 0.45^{b}	5.72 ± 0.32*	4.63 ± 0.50
Uterine horn (cm)	12.55 ± 1.50^{a}	6.46 ± 1.21^{c}	10.07 ± 1.60^{b}	6.00 ± 0.73^{cd}	-	-
Histological aspects of uterine artery during breeding season (November-April) and non-breeding (May-October)						
Diameter (µm)	250.01 ± 13.20^{a}	241.30 ± 10.20^{b}	233.30 ± 14.30^{c}	222.00 ± 11.30^{d}	245.7 ± 10.10*	227.30 ± 13.80
Tunica intima (µm)	9.90 ± 2.10^{a}	8.80 ± 1.20^{a}	12.00 ± 1.30^{a}	9.60 ± 0.90^{a}	9.35 ± 1.30	10.80 ± 1.10
Tunica media (µm)	240.40 ± 13.10^{a}	200.30 ± 23.40^{b}	200.20 ± 17.90^{b}	180.60 ± 21.40^{bcd}	220.35 ± 21.30*	190.40 ± 13.10
Elastic fibers (%)	33.40 ± 3.60^{b}	32.30 ± 2.70^{b}	30.50 ± 1.90^{bc}	39.60 ± 2.70^{a}	32.85 ± 3.30	35.05 ± 1.10*
Collagen fibers (%)	24.10 ± 1.50^{d}	25.30 ± 1.90^{cd}	29.10 ± 2.00^{b}	31.60 ± 2.10^{a}	24.70 ± 1.90	30.25 ± 2.50*
Smooth muscles (%)	22.10 ± 1.80^{c}	23.20 ± 2.50^{cd}	30.50 ± 1.90^{ab}	33.20 ± 1.10^{a}	22.65 ± 3.20	31.85 ± 4.20*
Intra-mural venules (µm^{2})	53.30 ± 3.30^{a}	35.50 ± 5.30^{c}	43.30 ± 4.40^{b}	36.30 ± 3.30^{c}	44.40 ± 9.10*	39.80 ± 8.20
Mast cells distribution (MC mm^{-2}) in arterio-venous complex during BS and NBS					2.30 ± 1.10	3.70 ± 1.50*

Means with different letters or * in a row are statistically different at $p < 0.05$.

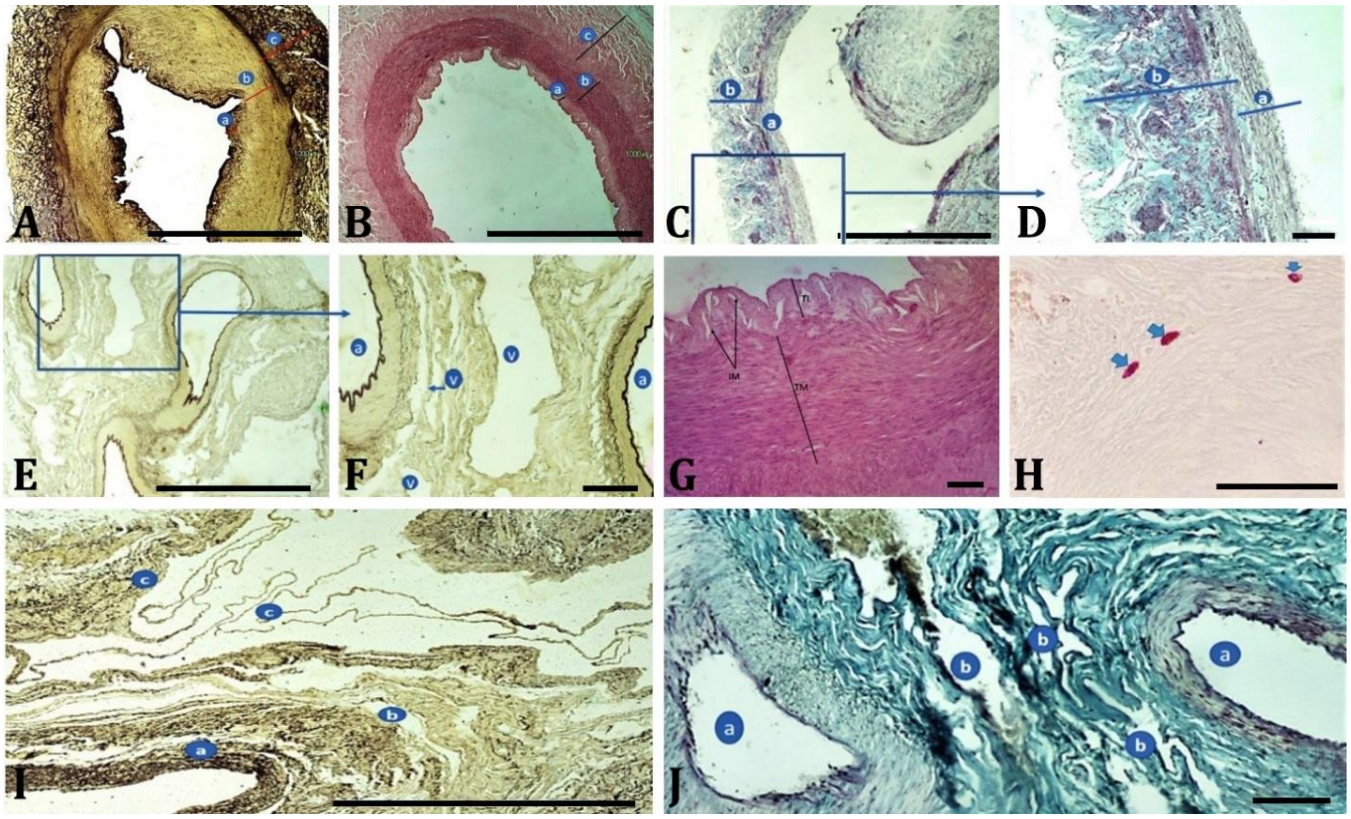

Fig. 1. A and B) Ovarian artery before entering the ovary notice the well-developed wall; a: tunica intima, b: tunica media, c: tunica adventitia (Weigert's elastic and H&E staining, respectively; scale bars = 1000 µm). **C and D)** Ovarian vein, notice the wall thickness with very little muscle cells; a: tunica intima; b: tunica media; c: tunica adventitia (Masson's Trichrome staining. scale bars=100 µm). **E and F).** Two adjacent ovarian arterial branches (a) and several veins (v) in between the two. (Weigert's elastic staining. scale bars = 100 µm). **G)** Ovarian artery showing tunica intima (TI) and tunica media (TM) containing smooth muscle fibers. A large number of intramural venules (IMV) in TI is present. (H&E staining. scale bar = 100 µm). **H)** Mast cells (blue arrows) identified in the arterio-venous complex (Toluidine Blue staining, scale bar = 1000 µm). **I)** Ovarian venous plexus, a: thick wall vein; b: thin-wall veins; c: valve. (Weigert's staining. scale bar = 1000 µm). **J)** Histological section of arterio-venous complex. Artery and veins are so close to each other and sharing same tunica adventitia composed of collagen fibers (Blue). Branching ovarian artery and veins in between the two vessels; a: branches of ovarian artery; b: branches of ovarian veins (Trichrome staining, scale bar = 100 µm).

Table 2. Serum level of hormones during different reproductive phases of female camels.

Hormones	Peak NBS-Extreme reproductive inactivity[1]	Start of BS-Start of reproductive activity[2]	Peak of BS-Peak of reproductive activity[3]	Start of NBS-Declining of reproductive activity[4]	Overall Mean	
					BS	NBS
Estrogen (pg mL^{-1})	21.33 ± 2.66^{c}	55.60 ± 1.90^{ab}	63.40 ± 4.82^{a}	40.34 ± 19.17^{b}	51.88 ± 9.27*	38.4 ± 3.72
Progesterone (ng mL^{-1})	0.17 ± 0.07^{b}	1.10 ± 0.00^{ab}	0.12 ± 0.07^{b}	0.196 ± 0.02^{b}	0.162 ± 0.04*	0.34 ± 0.16
Cortisol (ng mL^{-1})	50.50 ± 3.18^{a}	42.77 ± 2.93^{b}	34.03 ± 1.89^{c}	31.42 ± 2.78^{c}	32.73 ± 2.31	46.63 ± 3.73*

[1]: June-August, [2]: September-October, [3]: November-February, and [4]: March-April. BS: Breeding season, NBS: Non-breeding season.
[abc] Means with different letters or * in a row are statistically different at $p < 0.05$.

The changes in the pattern of serum progesterone level were seen as statistically non-significant ($p > 0.05$) during different phases of BS. The stress hormone, cortisol, was found inversely related to reproductive activity of the BS as it measured highest (50.50 ± 3.18 ng mL^{-1} in peak NBS-extreme reproductive inactivity phase (June-August) and lowest in peak BS reproductive phase (November-February) of the cycle. The cortisol level was positively related to THI determined during different months as given in the Table 2.

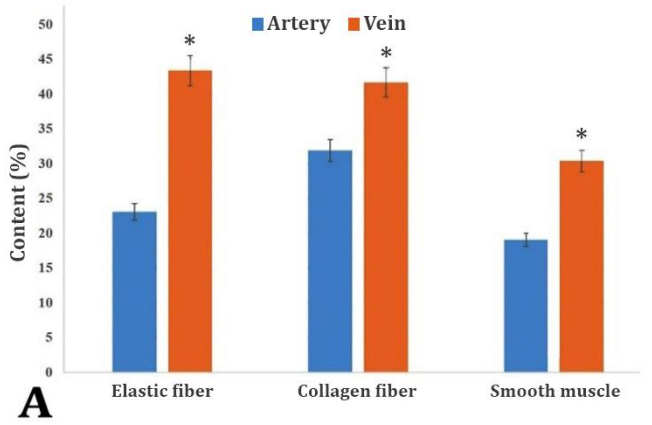

Fig. 2. A) Graphical representation of over all means of the fibrous components in ovarian artery and ovarian veins before entering into the arterio-venous complex. Asterisk indicates statistically significant difference at $p < 0.05$. **B)** Photomicrograph of ovarian artery. Represented the tunica intima (ti) of the artery containing numerous small-sized intra-mural venules (IMV). Smooth muscle can also be seen in the tunica media (tm), (H&E, scale bar = 100 µm).

The overall OA (left and right) diameter was recorded to be maximum in breeding season and minimum in the non-breeding season. The coefficient of correlation "R" was determined -0.86 and -0.40 for diameter, THI and cortisol, respectively. These values indicated a negative relationship of diameter, THI with cortisol level (Fig. 4C). Similarly, THI adversely affected the estrogen and progesterone level but correlation coefficient was recorded positive for cortisol during different phases of the breeding year (Table 2, Fig. 4D).

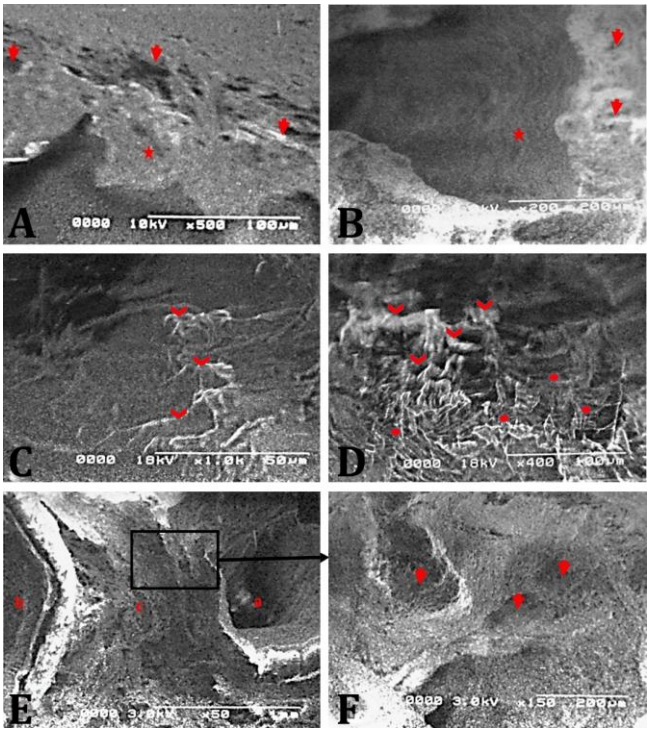

Fig. 3. Scanning electron micrograph (SEM) of transverse section of ovarian artery before entering into the arterio-venous complex in **A)** breeding and **B)** non-breeding season. Small red arrows represent the intramural venules area, which was observed more in the breeding season. Small red color stars indicate the endothelial cells of the artery. **C** and **D)** SEM of tunica adventitia of ovarian artery showed small-sized venules consists of anastomosing pattern wraps and penetrates the tunics of artery (red arrowheads). These small venules have very thin fibrous wall that may be helpful for the local transport of different substances (red stars). **E** and **F)** SEM of arteriovenous complex exhibited artery (a) and vein (b) showed the sharing of the same external tunic (c) making it difficult to differentiate the boundary of these vessels.

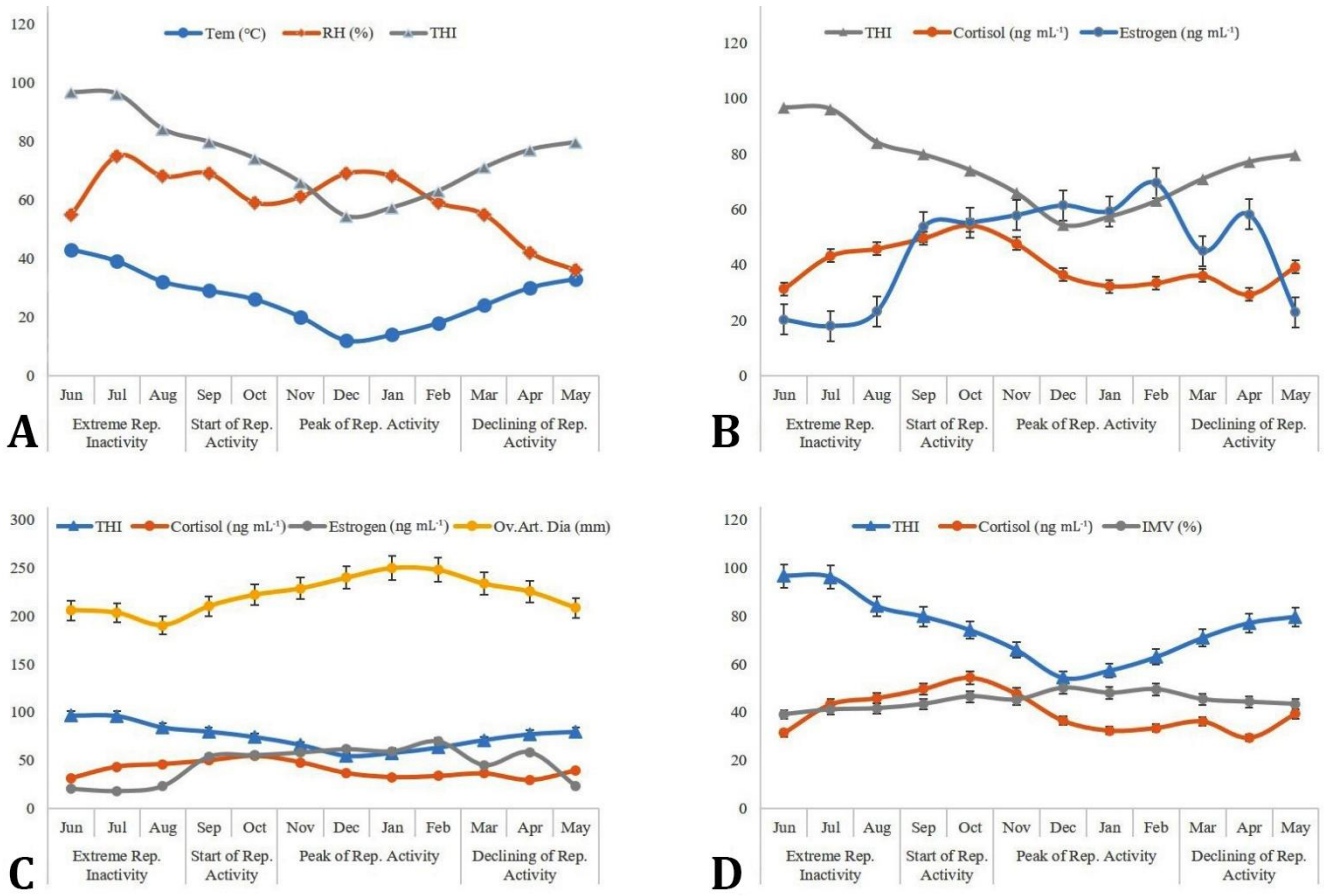

Fig. 4. Relationship of thermal-humidity index (THI) with temperature (Temp) relative humidity (RH), hormones and ovarian artery (OA) parameters during different phases of breeding cycle in one-humped camel. **A)** THI depends upon and directly related to Temp and RH and estimated highest in the months of June to August (extreme reproductive inactivity). **B)** Highest estrogen level was observed in winter (November-February) when THI is minimum while cortisol showed direct relation with THI and inverse with estrogen. **C)** Highest THI and cortisol caused decreasing of ovarian artery diameter (Ov. Art. Dia) and estrogen displayed positive relation with Ov. Art. Dia. **D)** Intramural Venules (IMV) followed the inverse trend with THI and cortisol.

Discussion

The vasculature of the female reproductive system in mammals (cow, sheep and swine) is highly adapted to prevent the detrimental effects of thermal stress and hormonal exchange within the reproductive system. Female one-humped camel has unique reproductive anatomical features like asymmetrical uterine horns, absence of inter-cornual ligament. In cow and swine extensive wrapping of the ovarian vein (OV) by branches of the ovarian artery (OA), sharing of same tunica adventitia by OV and OA and counter-current mechanism of ovarian steroidal hormones has been reported,[17] however, thermoregulatory role of these structure in camel are not studied yet. It is necessary to have an understanding of heat stress as it curtails reproductive performance by disturbing the granulosa cells activity, plasma levels of ovarian hormone and LH receptor.[18] Therefore, the undertaken study in camel is the first report that describes the thermal and seasonal influence on the gross and microanatomy of the reproductive system along its vasculature in relation to thermoregulation.

Camel is a seasonal breeder and shows reproductive activity in a specific period of the year in which plenty of food available in ambient temperature[19] The breeding cycle of the camel is divided into four different physiological phases including extreme reproductive inactivity (June-August), start of reproductive activity (September-October), peak of reproductive activity (November-February), declining of reproductive activity (March-April).[20] Reproductive system of female camel, like other mammals, comprises of ovaries, oviduct, bipartite uterus, cervix, vagina and external genitalia which are greatly influenced by the season.[10]

The gross morphological parameters including width, circumference and weight of ovaries, the diameter of oviduct and length of uterine horn significantly increased in BS. The size of left uterine horn is almost twice the size of right horn but during BS this difference becomes more significant. Ali *et al.*[21] and Srikandakumar *et al.*[10] described

similar findings but they did not link these changes with reproductive vasculature under the seasonal influence. This increase in ovarian and uterine gross parameters under the influence of season may result in vascular changes. Blood flow to the specific organ depends upon its metabolic needs. Larger diameter of the ovarian artery (OA) and close association of OV in BS may provide more area for supplying hormones and nutrients. This exchange of hormones and nutrients promotes ovarian folliculogenesis and uterine growth that results in increased parameters.

Histologically the medial layer of arteries is composed of principal components; smooth muscle, elastin and collagen fiber. The amount and fraction of these components and the wall organization varies with the type and anatomic location.[22] Results indicated that tunica media, collagen, elastic and smooth muscle contents varied among the different seasons as well as side of the reproductive system. No contemporary literature is available that describes the histology of these vessels in camel. The main arterial and venous portions of the ovarian vessels have regular vascular tunics in respect to the tunica media thickness. The possibility of exchange of heat and hormones is slim to none at this specific region of arterial and venous blood vessels encountered. However, as the vessels branches down approaching the ovary, the thickness of both arterial and venous portions showed much thinner walls in all three tunics. Zócalo *et al*.[23] described that these components and vasa-vasorum of any vessels were responsible for the energy dissipation among the system. The significant thicker tunica media (240.40 ± 13.10 µm) of left side vessel in BS was probably to accommodate more blood volume due to increase in diameter.[24]

The collagen and elastic fibers contents of vessels are inversely related to the pressure compliances that can be linked to larger diameter of OA to accommodate blood supply. Similarly, fiber contents and vasa-vasorum were directly related to the counter current mechanism for heat transfer as vasa-vasorum resulted in increased surface area of different tunics of vessels.[5,20] These features are observed more in OV. The smooth muscle contents with relation to heat exchange have not studied yet. However, Bia *et al*. [22] reported that the distribution of arterial blood throughout the body stimulates smooth muscle-dependent energy dissipation to cushion the pressure and strain stress.

A unique venous network is identified in tunica intima and media of OA. This new structure named intra-mural venules (IMV) was first described in the spermatic artery of bulls and considered responsible for hormonal and heat exchange in blood supplying the testes.[25] An extensive network of anastomosing venules and fibers was seen in the tunics of the OA before entering into the AVC. The deep penetration of these thinned walled venules into the walls of the OA may form IMV. No literature is available that describes ultrastructural studies of the reproductive vasculature of female camels. These thinned walled IMV in camel OA may be purposed as a morphological structure that has a role in temperature and hormonal exchange between arterial and venous blood. The area of IMV may be regarded as an anatomical site for effective local transfer of local heat and hormones that regulate organ function which was monitored larger in peak of the reproductive phase for greater exchange of different materials.[26]

Histologically, the AVC presented very close association of different tunics of vessels that sometimes were difficult to differentiate. Albeit, same kind of structure is present and explained by Tabęcka-Łonczyńska *et al*.[27] , Skipor *et al*.[28] and Stefańczyk-Krzymowska *et a.l*[29] and Koziorowsk[30] in swine and cow. But there is no scientific literature available that explains microscopic anatomy of reproductive vasculature of female camel. This AVC may be contemplated as analogous structure to pampiniform plexus present in males to cool arterial blood in adjacent arteries and veins, blood flows in opposite directions. The mast cells distribution in the AVC was seen significantly increased in the NBS. Mast cells are composed of granules that upon activation degranulation stimulate inflammatory activity. In testes, mast cells directly involved in fibrotic activity that severely effects the male fertility. [31] More number of mast cells in the NBS, may be involved in the fibrosis that consequently thickened the thinned walled vessels and hampers the transport of different substances within the AVC. The concentration of hormones and temperature in venous blood from the ovary is, on average, 10- to 20-fold greater than that in the systemic arterial blood entering the OA.[32] This difference creates a concentration gradient that enables heat and hormone to exchange from the branches of the ovarian vein into the branches of ovarian artery by a counter-current heat exchange mechanism. Bendz[33] demonstrated morphological evidence for a similar vascular anatomy in humans as in the sheep. In forty-three humans, adnexa were studied, tortuous arteries were found in close contact with veins. Microscopic sections revealed thinning of the vein walls at contact areas.[34] The presence of valves within the veins will hinder the blood return from the ovary and or uterine horn and thus may facilitate the passage of hormones or prostaglandins from one vessel to the other through creation of a concentration gradient.

Being a seasonal breeder, the ovarian activity of camels is boosted in BS in terms of follicular growth and development. Like Skidmore[12] and Manjunatha *et al*.[35] the highest value of estrogen (63.40 ± 4.82 pg mL^{-1}) was detected in months of peak BS as compared to NBS-extreme reproductive inactivity (21.33 ± 2.66 pg mL^{-1}). This upsurge in estrogen level is consequence of increased granulosa cell proliferation, estrogen secreting cells, in

breeding season.[21,36] Larger ovarian artery diameter in breeding season may be attributed to higher levels of its estrogen content which is known to have vasodilator activity through endothelial production of nitric oxide [37] and inhibition of smooth muscle proliferation by impeding the mitogen-activated protein kinas.[38] Apart from ovarian artery diameter, IMV have also shown positive correlation (R=0.88) with estrogen level which may act as a site for local estrogen diffusion from artery to vein.

Serum progesterone level remained low (< 1.00 ng mL^{-1}) throughout the year and did not show any significant ($P < 0.05$) correlation to season and THI. Kamoun and Jemmali[39] recorded similar observations in female camel. Camels are induced ovulators, they require mating by the male partner for ovulation that leads to the formation of corpus luteum (CL). Progesterone is primarily secreted from luteal cells of the CL. No CL on ovaries was seen in BS which was why low levels of progesterone were recorded. Under the influence of progesterone, core body temperature rises about 0.50 to 2.00 ˚C because of the thermogenic property of progesterone.[40] Heat conservative mechanisms like vasoconstriction are promoted by progesterone but exact mechanism is still vague especially in camels. Countercurrent diffusion of this hormone in the vasculature of the reproductive system in relation to body temperature needs to be investigated. Serum cortisol level was recorded the highest (50.50 ± 3.18 ng mL^{-1} June, July and August which were in line with the observation of Zia-Ur-Rahman *et al*. [41] in males and Shujait *et al*.[36] in female camels. High temperature and relative humidity cause an increase in stress indicator, THI, which might have led to higher cortisol levels.

High cortisol levels inhibit nitric oxide synthase which is a strong vasodilator.[42] Smaller diameter of OA in NBS could be due to nitric oxide inhibition by cortisol level. The smaller the OA diameter, the lesser will be the area available for the local transfer of hormones and heat within the reproductive system. Suboptimal transfer of hormones and heat in NBS could be related to a decrease reproductive activity and morphological parameters of the ovary and uterus. Intra-mural venules were also found negatively correlated to cortisol level but the underlying mechanism behind this phenomenon needs to be explored on molecular level.

It is hypothesized that counter-current heat exchange, certain hormones and/or prostaglandins may cross over in female camel as it was demonstrated by other researchers in other species. In ruminants, prostaglandin F2α, estrogen and progesterone are synthesized and released in a pulsatile pattern. The unique structure of the vascular utero-ovarian plexus allows the transport of these hormones directly from the uterus to the ovary bypassing the systemic circulation. However, the underlying molecular mechanism is not known.[43]

Hormonal, seasonal, stress indicator and vascular dynamic of the female genital system are interlinked and IMV along with the close association of OA and OV may be considered as the site of countercurrent exchange in the reproductive system of female camel.

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Conflicts of interest

Authors declared no conflict of interest related to the data used in this manuscript.

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