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Seasonal histological and Morphometrical changes in the Testis of Adult Awassi Ram: Angiogenesis

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Abstract
The objective of this study was to focus the light on the events of angiogenesis during spring and summer times. For this purpose, testes of ten rams were collected from Baghdad in spring and summer seasons in 2017.Samples were histologically processed and prepared for light microscopy. Hematoxylin and eosin stain was used for staining. The results revealed that the process of angiogenesis was took place during studied seasons, summer and spring. The onset of angiogenesis occurred firstly during spring and then lasted during summer; however it is more prominent in summer time. The diameters of capillaries in spring and summer were similar, while diameters and thickness of arterioles were larger in summer compared to spring. The testicular interstitial tissue in summer time was (24%) represented more than twice that of spring time (11%). The physiological increase in testicular vascularization was more than 2.5 fold in summer time (9.5%) compared to spring time (3.7%). The diameter and thickness wall of arterioles in summer time was higher than in spring. The interstitium and its constituents were more in summer time than in spring time. The study revealed the presense of close relationship between the pre-existing endothelium and pericytes to achieve the events of angiogenesis. These two cell types were mostly involved in the process of angiogenesis. The present study hypothesized that the event of angiogenesis was thrown into four steps.

Keywords: Ram, Testes, Angiogenesis

1. Introduction
The angiogenesis had been well studied in developmental events and pathological conditions; but the role of angiogenic factors in mature blood vessels is not well known [1]. Physiological angiogenesis in the adult mostly restricted to female and male reproductive tract during gonadal recrudescence in seasonal breeder animals [2, 3].
Angiogenesis can be contributed by cell division of pericytes, which are able to be transformed into fibroblast and smooth muscle cells. Cell processes of pericytes can contact with capillary endothelium [4]. Lissbrant [5] stated that endothelial cell proliferation was higher in male adult reproductive organs such as the testes. A continuous turnover of endothelial cells, accompanied by apoptosis has been reported in male reproductive organs under normal conditions [1]. Pericytes share the basement membrane of the endothelial cells. They undergo differentiation to become smooth muscle cells and endothelial cells in the walls of arterioles and venules. They aid in the angiogenetic processes and protect the endothelial cells from anti angiogenic therapies [6]. Aughey and Fry [7] reported that pericytes are pale staining C.T cells lying adjacent to the capillary endothelium. The endothelial cells and pericytes retaining the capacity to divide and to synthesize collagen and extracellular ground substance. They are differentiated and can give rise to both fibroblasts and smooth muscle cells in areas of tissue repair and revascularization and repair of damaged blood vessels. Davidoff *et al.* [8] mentioned that pericytes aid in maintenance, physiological repair, and regeneration of organs under local environmental factors. It has been suggested that Leydig cells secrete angiogenic factors [1]. Mayerhofer [9] referred to the changes in the testicular microvasculature during photoperiod-related seasonal transition from reproductive quiescence to reproductive activity in the adult Golden Hamster.

2. Materials and methods
The objective of this study was to focus the light on the histological events of angiogenesis in the testes of Rams. For this purpose, ten adult Awassi Rams were collected from Baghdad in 2017.

The study was carried out in Department of Anatomy and Histology, College of Veterinary medicine, University of Baghdad, Baghdad, Iraq. Five samples during spring season (March & April) and the same number during summer season (July & August). The environmental temperatures were measured in both seasons. They were 29℃ in spring and 49℃ in summer. Routine histological processes were carried out to prepare specimens for light microscopy. Hematoxylin and eosin stain was used for staining [10]. Several morphometric parameters were studied during the two seasons which include: diameter of different blood vessels, diameter of endothelial cells, and diameter of myocytes, thickness of the wall of blood vessels, ratio of tubular tissue to interstitial tissue and ratio of blood vessels to the interstitial tissue [11]. Color USB 2.0 digital camera (Scope Image 9.0) was used to achieve the micromorphometric measurements which is provided with image processing software connected to light microscope.

2.1. Statistical analysis

Computer package (Sigma plot V12.0 / SYSTAT software) was used to conduct the histomorphometrical analysis. Data were presented as means ± SE (standard error) and were analyzed using T-test with significant level set on P <0.05. The differences among the groups were determined by Duncan's multiple range tests [12].

3. Results

The present study revealed that the process of angiogenesis was took place during studied seasons, summer and spring. The present study revealed that the amount of testicular interstitium, the testicular blood vessels, the endothelial cells, and the myocytes were predominated in summer time than in springtime. The onset of angiogenesis was took place firstly during spring and then lasting during summer; however it was more prominent in summer time. Different sizes of newly formed capillaries and cells were observed. The diameters of capillaries in spring and summer were similar, while the diameters and thickness of arterioles in summer were larger than spring (Table 1, 2). The endothelium supplies the newly formed tunica intima with squamous cells which having curved dark nuclei. Tunica adventitia had smaller flattened dark nuclei. Pericytes provide tunica muscularis with large number of newly formed euchromatic smooth muscle cells with 1-2 large obvious centrally or eccentrically located nucleoli. Events of cellular mitosis, protein synthesis, and transformation were noticed (Fig. 1, 2, 3). During seasonal stimulation, the dormant squamous cells of tunica intima of the pre-existing testicular blood vessels were activated; their nuclei were transformed from the quiescent heterochromatic state to the activated euchromatic state. Their nuclei were changed from dark flattened to pale ovoid state with their chromatinous network became more obvious (Fig.1). These nuclei were enlarged and bulged into the lumen of the blood vessel in a configuration that gives the arteriole the clock-face appearance (Fig. 4, 5). In tunica muscularis, large number of newly formed small cells in which pale spherical or ovoid nuclei were noted with 1-2 large apparent eccentrically located nucleoli (Fig.1). Different sizes of newly formed capillaries and cells were observed (Table 1). The study concluded that, during seasonal stimulation, two cell types can share the formation of newly formed blood vessels, i.e., the squamous cells of tunica intima which can play an important role in the formation and development of newly formed blood capillaries, and the pericytes that can be differentiate and modified to myocytes. The present study hypothesized that the event of angiogenesis was thrown into four steps, namely:

1. The step of cellular proliferation, in which the endothelial cells and pericytes were simultaneously proliferate.
2. The step of cellular arrangement, in which:
   a- The dividing endothelial cells supply both the tunica intima and tunica adventitia with curved endothelial cells to achieve the tube formation of the blood vessels.
   b- The dividing pericytes provide the tunica media with smooth muscle cells.
3. The step of ground substance formation, in which the cells secrete the needed fibers and substances.
4. The step of attachment of the newly-formed blood vessels to the pre-existing ones (Fig.4).

Table 1: Micromorphometric measurements of interstitial tissue components of ram in spring and winter seasons/ µm.

Parameters	season	
	spring	**summer**
diameter of arterioles µm	28.13± 1.3 a	39.07± 0.7 b
diameter of capillaries µm	9.7± 0.3 a	9.6± 0.4 a
diameter of endothelial cells(arteriole) µm	2.7± 0.05 a	3.68± 0.1 b
diameter of myocyte(arteriole) µm	4.02± 0.08 a	5.06± 0.2 b
thickness of arteriole wall µm	10.71± 0.4 a	14.21± 0.9 b

The different letters in columns means there is a significant difference (p<0.05) among age's

Table 2: Percentage of interstitial tissue and blood vessels in the testes of ram in spring and winter seasons.

Parameters	season	
	spring	**summer**
%of interstitial tissue in testis	11%	24%
% of BV in the interstitial tissue	3.70%	9.50%

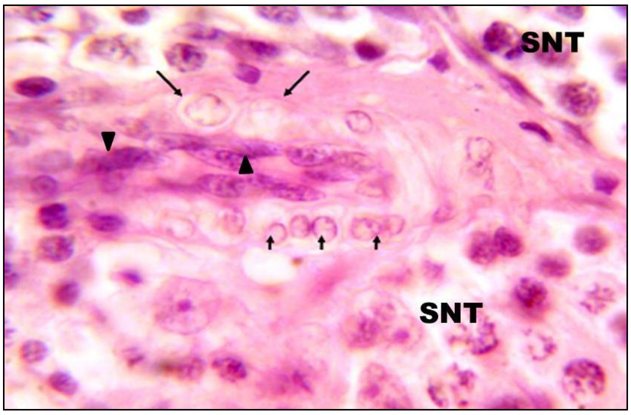

Fig 1: Micrograph of testis of adult ram in summer time showing blood-filled arteriole lies between two seminiferous tubules (SNT) in the process of angiogenesis. Notice the stimulated well developed endothelium with large oval euchromatic nuclei and clear nucleoli (arrow head). Long arrows refer to non- dividing pericytes at the periphery of the arteriole. Short arrows indicate the smaller smooth muscle cells that arise from dividing pericytes. 400X. H & E stain.

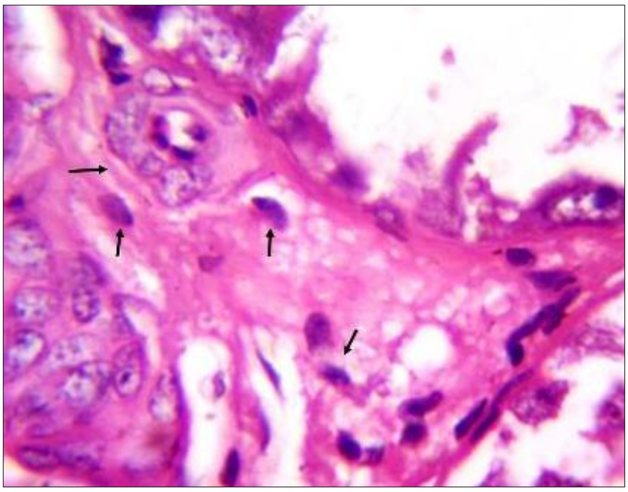

Fig 2: Micrograph of testis in adult ram showing different steps of the process of angiogenesis in the interstitial tissue in summer season (arrows). 1000X. H & E stain.

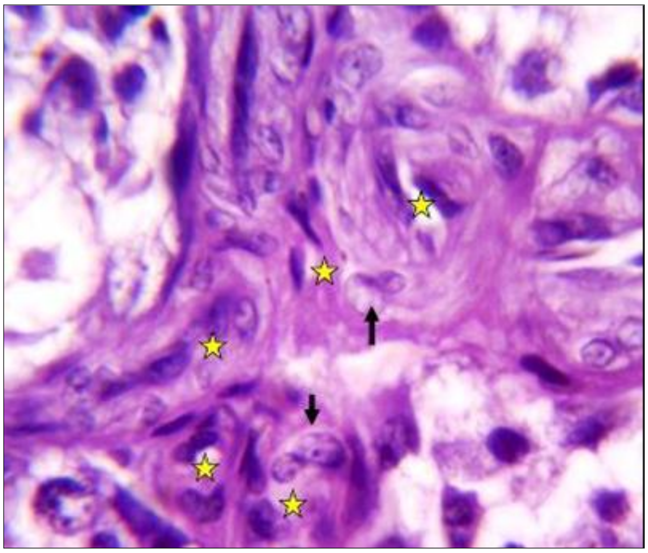

Fig 3: Micrograph of testis in adult ram in summer season showing the development of different sizes of blood vessels (stars). Arrows refers to the pericytes. 1000X. H & E stain.

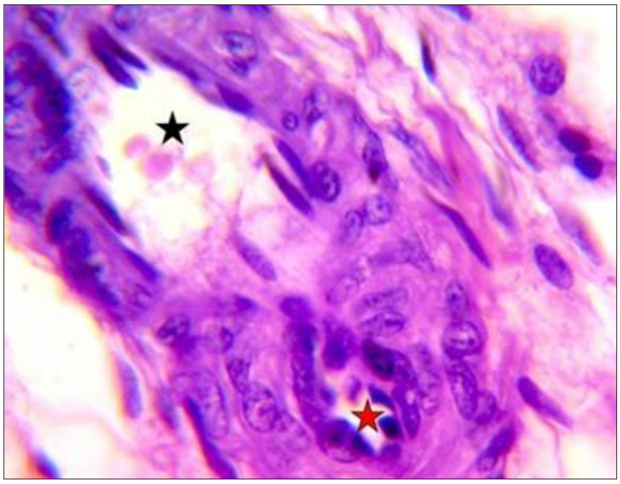

Fig 4: Micrograph of testis of adult ram in summer season showing newly formed arteriole (red star) attached with pre-existing arteriole (black star) to connect the blood stream of the two. 1000X. H & E stain.

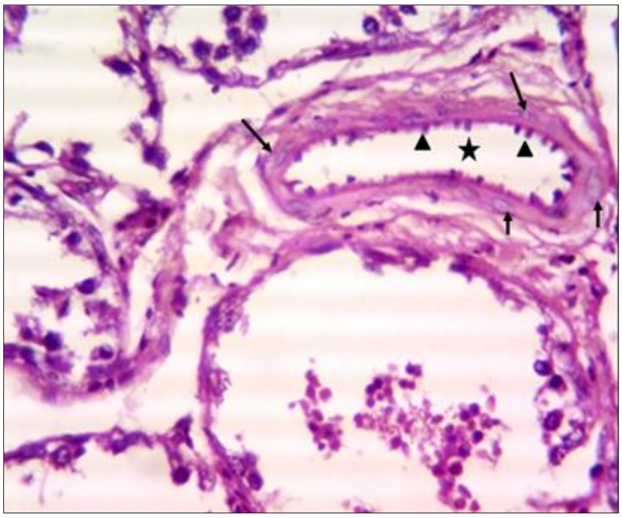

Fig 5: Micrograph in the testis of adult ram in spring. Star refers to venule with bulging nuclei of endothelium (arrow head). Note the dividing (long arrows) and non-dividing (short arrows) pericytes. 100X. H & E stain.

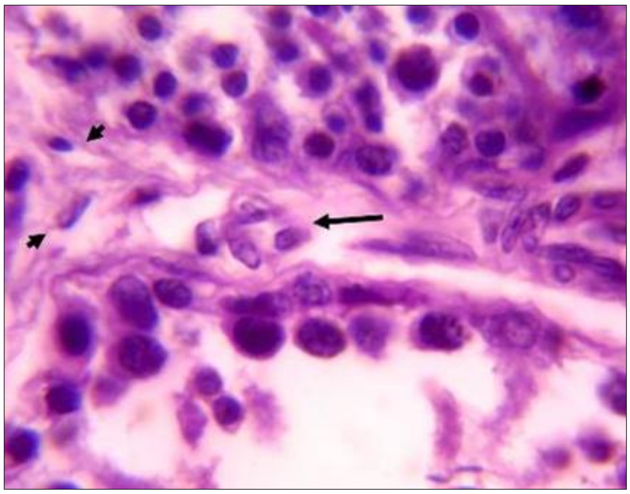

Fig 6: Micrograph of the testis of adult ram in spring season. Process of angiogenesis in arteriole (long arrow) and two capillaries (small arrows). 1000X. H & E stain.

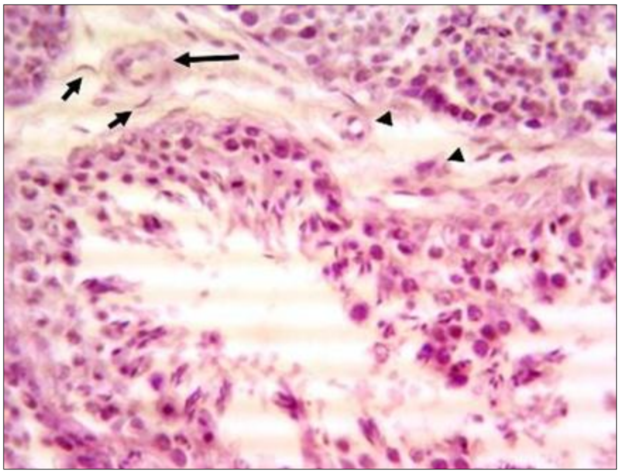

Fig 7: Micrograph of the testis of adult ram in spring season. Small arterioles (arrow heads) and large arteriole (long arrow). Note the curved endothelial nuclei of the arteriole in the process of angiogenesis (small arrows). 400X. H & E stain.

4. Discussion

The present study revealed that the great variation in environmental temperatures between summer and spring in Iraq was positively affected the process of testicular angiogenesis. This explains the increase in the percentage, diameters and thickness of the blood vessels, as well as, the diameter of nuclei of the endothelial and pericytes in the interstitial tissue in summer time compared to spring time. These morphological changes being in accordance with those found previously in camel by Pasha *et al.* [13], Zayed *et al.* [14], Pasha b *et al.* [15], Tingari [16] where they concluded that the seasonal anatomical changes (macroscopic, microscopic, ultra structural, and ultrasonography), in the camel's testis were well established and found that the seminiferous tubule diameter was comparatively reduced, and the interstitium occupies a larger area during the rutting season of camels.

The findings of the present study was similar to that of Pyter [17] in rodents who suggest that adult male rodents display seasonal angiogenesis to support seasonal changes in reproductive tissue morphology and agreed with Mayerhofer *et al.* [9] who referred to the increase in angiogenesis in hamster testes for physiological recrudescence.

Abdel-Raouf *et al.* [18] and Marai *et al.* [19] believe that the diameter of the seminiferous tubules become larger during the rutting season, and tends to be smaller in the non-breeding season of the camel. This study postulated that summer time was the season of preparation, while spring time was the season of activity, secretion and cellular movement. Endothelial cells and pericytes were the most cells which were involved and stimulated in the process of angiogenesis. This was in agreement with Davidoff *et al.* [8] who referred to the role of pericytes on physiological states under local environmental factors; similarly, Mayerhofer *et al.* [9] declared that physiological angiogenesis occurs in the testes during testicular recrudescence in adult golden hamster. The previous authors referred that testes were involved in an increase in densities of testicular blood vessels in spring season; this was in variance with the findings of the present study in which the angiogenesis in summer time was more apparent than spring time. The current study was in agreement with Baker and Witt [20] who stated that pericytes were versatile cells in the blood vessels. They proliferate, grow easily, and differentiate into other ectodermal and endodermal cells. The presence of different sizes of newly formed capillaries monitored the developmental formation of new arteriole or venule. The presence of different diameters of cells indicates also that new events of tissue formation, modification, and development were took place. This was in accordance with Hellstrom *et al.* [21] concerning the role of pericytes in blood vessel formation. The present results were confirmed with the findings of Berger and song [22] who demonstrated that pericytes can sense angiogenic stimuli, guide the newly formed tubes and elicit endothelium. After seasonal stimulation, the development of the nuclear chromatinous materials of the endothelium indicates that these cells were going toward cellular division. The endothelial cells were enrolled up to form a new capillary tube. This was confirmed by Mesher [23] and Franco *et al.* [6] who reported that after any stimulation, injury, and repair, Pericytes can proliferate to form smooth muscle cells when a capillary is transformed into an arteriole or venule and protects the endothelial cells from any anti angiogenic effect. The endothelial cells respond to blood flow changes, stretch, and a variety of circulating substances. They secrete growth factors and vasoactive substances [24]. Greenhalgh [25] reported that macrophages are stimulated by decrease oxygen concentration of their surroundings to produce factors that induce and speed angiogenesis. The current study was in accordance with Alexandre-Pires *et al* 2012 [26] hypothesized that the increase in testicular vascularization might be related to higher seasonal sexual activity in cats which is in agreement with the fact that most queens give birth at the beginning of the year, between May and July and in September.

5. Conclusion

It was concluded that summer season elicits strongly both the innermost endothelial cells and the outermost peripheral pericytes to share in the process of angiogenesis. The present study hypothesized that the event of angiogenesis was thrown into four steps; cellular proliferation, cellular arrangement, ground substance formation and newly-formed blood vessels

6. Acknowledgment

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