

## Histomorphological study of the spleen in indigenous Gazelle (*Gazella subgutturosa*)

M. H. Abed Muslih and S. M. Mirhish

<sup>1</sup>Department of Anatomy and Histology, College of Veterinary Medicine, Baghdad University, Iraq.

E-mail: [shakirmirhish@gmail.com](mailto:shakirmirhish@gmail.com)

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### Summary

The study aimed to investigate the anatomical and histomorphometrical features of spleen in gazelle. To achieve this goal, spleens of 7 animals were used. The gross aspect of the study revealed that the spleen of gazelle appeared dark brown in color with elliptical shape and was situated at the left lateral surface of the rumen. It occupied the area extended from the 8<sup>th</sup> rib to 11<sup>th</sup> rib. Length and width of the spleens were calculated and the mean were  $7.94 \pm 0.1$ ,  $5.88 \pm 0.2$  respectively. There were three ligaments connected the spleen with adjacent structures, namely splenogastric, splenorenal and splenophrenic. The blood supply of the spleen was studied using X-rays and resin injection methods. The splenic artery was divided into three primary branches, each one subdivided into two secondary branches. The latter branched into tertiary branches which were distributed all over the splenic parenchyma. Microscopic findings showed that the capsule of spleen in gazelle was composed from of two layers, the outer one made of mainly of dense connective tissue; whereas the inner layer of capsule consisted of connective tissue fibers in addition to abundance of smooth muscle fibers interweaving among them. The total mean thickness of the capsule was  $210.51 \pm 8.3$   $\mu\text{m}$ . The splenic parenchyma in gazelle consisted of white pulp represented by lymphoid follicles with their marginal zones and periarterial lymphatic sheath while the red pulp represented by splenic cords and sinusoids. The area of white pulp in the gazelle comprised about 9.6% of the spleen parenchyma. Red pulp composed mainly from cords of connective tissue and small sinusoids or sinusoids filled with blood cellular element which extended among these cords and was lined by flattened endothelial cells with relatively large spaces or slits between them. The sinusoids were found to be lymphocytes, reticular, plasma, macrophages and occasional megakaryocytes whereas the megakaryocyte appeared large with acidophilic cytoplasm and dark elongated multilobated nucleus. This research work was performed in order to establish the basic histomorphological information helpful for the veterinary medical practice and veterinary surgeons to developing their work on gazelle like designing the approach of some surgical operations like splenectomy or any surgical entrance may be needed in these animal species.

**Keywords:** Gazelle, Spleen, Red pulp, White pulp, Periarterial lymphatic sheath.

### Introduction

Gazelle (*Gazella subgutturosa*) is found in northern Azerbaijan, eastern Georgia, part of Iran, parts of Iraq, southwestern Pakistan, southeastern Turkey, Afghanistan and in Uzbekistan. Large herds were also present in the Near East. Since 6,000 years ago, they were captured and killed with the help of desert kites (1). The spleen is triangular in shape covered by a thick fibrous capsule. Many blood vessels invaded the capsule that originated from splenic artery toward the parenchyma of spleen. The trabeculae extended from the capsule which contains blood vessels as branched from the splenic artery (2). Generally the spleen has two

surfaces; parietal convex and concave gastric (3). There were differences in shape, location and structures in different animal species and such anatomical differences reflects the different function of the spleen (4). The mammalian spleen composed from white pulp which presented by periarterial lymphatic sheath (PALS). The lymph follicle is predominant in the spleen of man whereas the PALS predominant in rat spleen. The reticular framework of white pulp is divided into two parts in mice and man which are the first part that surrounding the PALS and the second part that surrounding the follicles. In all vertebrates it was considered the major single mass of lymphoid tissue but the architecture design of

the spleen in domestic and wild animals was different in form and histological structure. These facts may be considered helpful guides to differentiate the spleen among different species in vertebrates (5). The aim of this study was to investigate the anatomical and histomorphometrical features of spleen in gazelle.

### Materials and Methods

This work was carried out on a total of seven spleens that were collected from the gazelles (*gazella subgutturosa*) Ksaiba Forest Reserve in Baghdad governorate. All animals in this study were clinically healthy males. The gazelle aged between 14 – 18 months and their weights ranged from 17 – 19 kg, the location and boundaries of spleens were determined in situ then dissected away from the abdomen of the animal and washed using buffered saline.

Gross anatomy observation included: Anatomical location, boundaries, attachments and ligaments. Shape and color measurements length with the thickness from middle and periphery and weight of spleen/ gm. Blood supply using X- rays latex and resin injection methods (Each spleen was perfused with normal saline through splenic artery.

**Table, 1: Gross measurements of spleen in gazelle.**

Parameter	Weight of animal (Kg)	Weight of spleen (mg)	Length of spleen (cm)	Width of spleen (cm)	Thickness of spleen from Highest area (mm)	Thickness of spleen from Lowest area (mm)	Thickness of spleen from Left side (mm)	Thickness of spleen from Right side (mm)
Animal								
Gazelle	17.8±0.4	30.3±5.4	7.94±0.1	5.88±0.2	13.75±1.5	2.64±0.4	3.74±0.4	3.97±0.2

It has two ends called cranial gastric and caudal intestinal end. The cranial end of the spleen is expanded, while the caudal end is rounded. The parietal surface of the spleen was convex with smooth appearance whereas the visceral surface was slightly concave and had gastric impression. The hilus is located on the spleen on the intermediate region of gastric impression from the hilum. The hilus of the spleen appeared as a longitudinal ridge (slit like shape) and represented the site of entrance of splenic blood vessels which divided the visceral surface of spleen into cranial gastric surface and caudal intestinal surface to contact the stomach and the intestine, respectively (Fig. 1). The splenic blood vessels were transmitted, and provided attachment to the

The samples were fixed using 10% neutral buffered formalin (NBF) for 72 hrs. Then they were processed by routine histological technique to get histological sections of 5-6 µm thick (6). Sections were stained with Harris's hematoxylin and eosin stain for general histological features and for micromorphometric measurements, the following stains were conducted Masson's Trichrome stain for demonstration of collagen and smooth muscle fibers. Toluidine blue stain was used for demonstration of marginal and mantle zones of the white pulp.

### Results and Discussion

The spleen of gazelle appeared dark brown in color with the elliptical shape, wide, expanded cranially and narrow rounded caudally (Fig. 1). It is situated at left lateral side of the rumen, the surface of the spleen was adjacent to the rumen called visceral or gastric surface at upper left quadrant of abdominal cavity and the other surface called parietal or diaphragmatic which face the musculature part of the diaphragm. The mean length and width were 7.94±0.1 and 5.88±0.2, respectively (Table, 1).

gastrosplenic ligament. The spleen was surrounded by peritoneum and suspended by many ligaments. The latter were three ligaments originated from the surrounding structures, that were splenogastric ligament (Fig. 2), splenorenal and splenophrenic ligaments (Fig. 3).

The blood supply of the spleen was provided by the splenic artery which branched from the celiac artery which is one of the branches of the abdominal aorta. Branches like the dorsal primary branch, intermediate primary branch and ventral primary branch were originated from splenic artery at the same level, each one of them was subdivided into two secondary branches and continued to give tertiary branches which were distributed all over splenic parenchyma (Fig. 4).

The capsule of the spleen appeared to be composed of two layers, the outer one consisted mainly of dense connective tissue; whereas the inner layer consisted of connective tissue fibers with abundance of smooth muscle fibers interweaving among them (Fig. 5). Micromorphometric measurements showed the total mean thickness of capsule was  $210.51 \pm 8.3 \mu\text{m}$ , out of which the thickness of the inner layer of capsule was  $116.7 \pm 4.1 \mu\text{m}$  (Table, 2). Microscopic examination showed the presence of peritoneal layer enclosing the fibrous capsule; this peritoneal layer was covered by single layer of mesothelial cells. (Fig. 6).

**Table, 2: Micromorphometric measurements of the stroma of the spleen.**

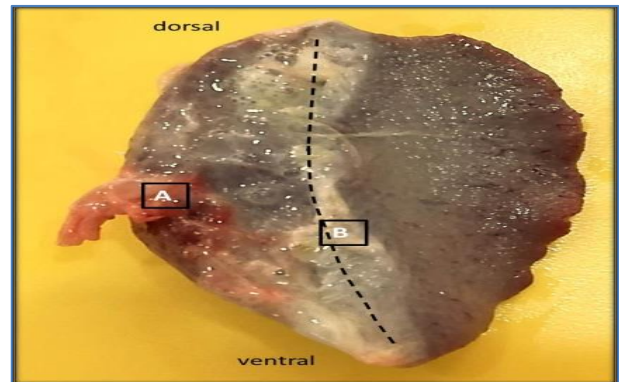
capsule thickness/ $\mu\text{m}$	Inner layer	Outer layer	Total
	$116.1 \pm 4.1$	$94.1 \pm 5.2\text{a}$	$210.51 \pm 8.3\text{a}$
Tr. thickness / $\mu\text{m}$	$97.46 \pm 5.4\text{a}$		
sub capsular space thickness / $\mu\text{m}$	$16.35 \pm 0.12\text{a}$		

The splenic trabeculae are extended from capsule into parenchyma, and composed mainly from connective tissue fibers with few smooth muscle fibers. The trabeculae carried blood vessels (trabecular arteries and veins) and nerves (Fig. 7) were surrounded by relatively large sinusoids called peritrabecular sinusoid (Fig. 7). The structure of the parenchyma was composed of white and red pulps. The white pulp was constructed mainly from lymphoid follicles with their marginal zones in addition to the Periarterial lymphatic sheath around central artery. The shape of the white pulp appeared rounded in shape with some others irregular in shape with narrow or ill-defined germinal centers; some sections were devoid of germinal center (Fig. 8). The central artery of white pulp was eccentrically situated in most studied sections, but in some other few sections found centrally located. Most white pulps contained one branch of the central artery, but few sections contained more than one branch; these current measurements showed that the area occupied by white pulp per  $1 \text{ mm}^2$  of spleen was  $0.096 \pm 0.003$  with surface area percentage of 9.6% only. The red pulps were composed mainly of cords of connective tissue extending and anastomose with each other in the parenchyma of spleen.

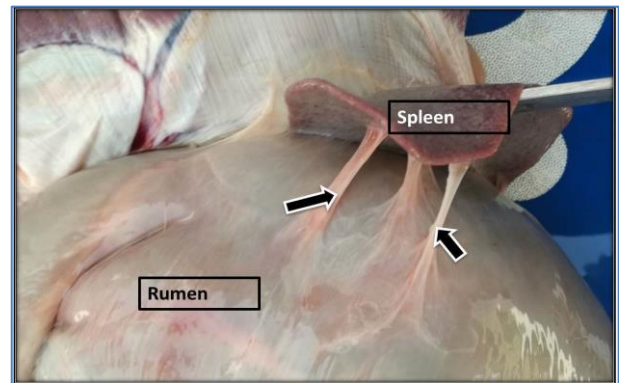
Small sinuses or sinusoids filled with blood cellular elements extending among these cords and lined by flattened endothelial cells with relatively large spaces or slits between them.

**Table, 3: Micromorphometric measurements of the whit pulp of the spleen.**

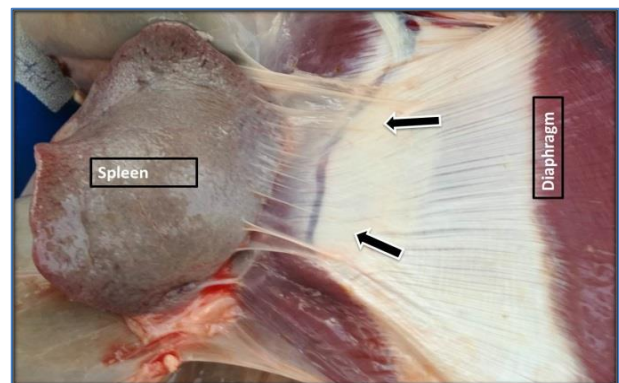
Parameter	Diameter of follicles / $\mu\text{m}$	G.center diameter / $\mu\text{m}$	marg.zone thikness / $\mu\text{m}$	PALS thickness / $\mu\text{m}$
Animal	mean $\pm$ SE	mean $\pm$ SE	mean $\pm$ SE	mean $\pm$ SE
Gazelle	$442.74 \pm 12.51$	$95.19 \pm 2.50$	$161.80 \pm 3.72$	$36.25 \pm 1.05$



**Figure, 1: photograph showing the visceral surface of gazelle spleen. (A) Hilus, (B) dotted line represent remnant of Splenogastric ligament.**



**Figure, 2: Shows the splenogastric ligament (arrows) of gazelle spleen.**



**Figure, 3: Shows the splenophrenic ligament (arrows) of gazelle spleen.**

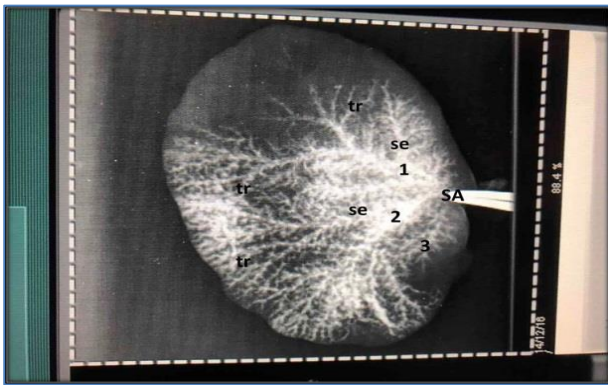


Figure 4: x-ray of gazelle spleen showing the distribution of splenic artery (SA). (1) Dorsal primary branch, (2) intermediate primary branch, (3) ventral primary branch, (se) secondary branch, (tr) tertiary branch.

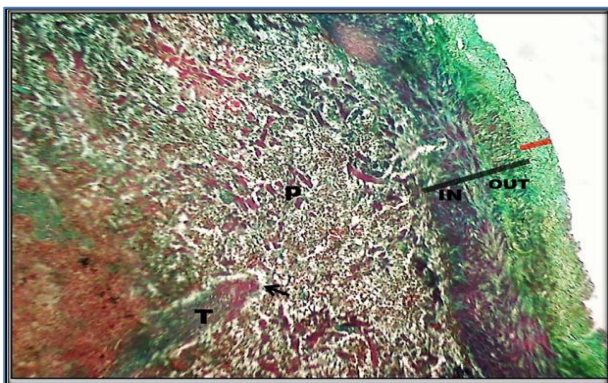


Figure 5: Gazelle spleen showing: capsule (black line), outer fibrous layer (OUT), inner muscular layer (IN), peritoneum (red line), trabeculae (T), peritrabecular sinusoid (black arrow) and splenic pulp (P). X100 Masson's trichrome stain.

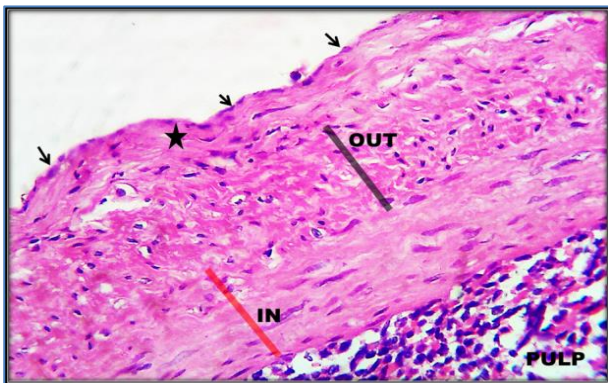


Figure 6: Capsule of spleen in gazelle showing: mesothelial cells (arrows), outer fibrous layer (black line), inner muscular layer (red line), peritoneum (black star) and splenic pulp. X400 H and E.

The spleen of gazelle appeared dark brown in color and elliptical shape, wide dorsally and narrow ventrally in contrary to other animal species such as bright red color (*Sus scrofa*) in Nigeria indigenous pig and West African dwarf goats light brown color (7). Present results were in agreement with those of (8), who described the camel spleen as dark brown in color and C-shaped with blunt and rough

edges. The shape, color, size and weight of the spleen varied according to the species (9). It was described that the cow has elongated organ and both extremities being thin, rounded and equal in size. The spleen in Gazelle was different from some carnivores animals such as cats and leopards. The spleens of cats and leopards were grayish, large, curved, flattened, elongated organ showing a narrow curved dorsal halve and wide almost straight part (10).

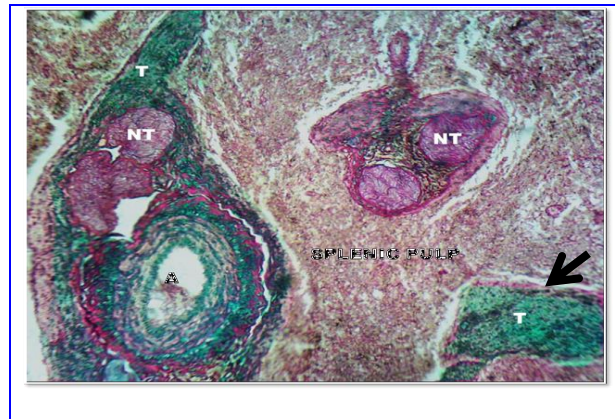


Figure 7: Gazelle spleen showing: Trabeculae (T) carry trabecular artery (A) and NT, peritrabecular sinusoid (black arrows). X100, Masson's trichrome stain.

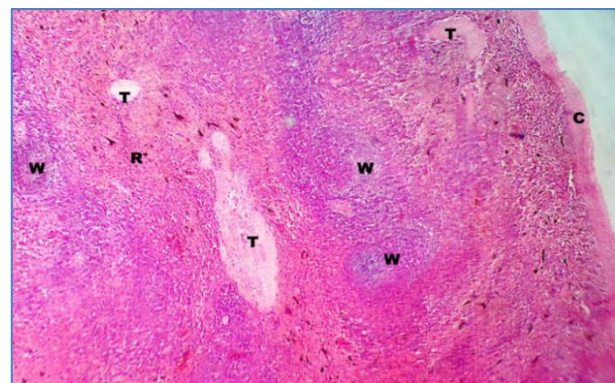


Figure 8: Gazelle spleen showing: capsule (c), trabeculae (T), white pulp (w), and red pulp (R). X H and E.

The spleen in all mammals is located in the abdominal cavity, directly beneath the diaphragm, and connected to the stomach (11). In gazelle the spleen was situated at the left lateral surface of rumen facing to the muscular part of the diaphragm and extended between the 8<sup>th</sup> rib and 11<sup>th</sup> rib. There were three ligaments connecting the spleens of gazelle with other structures which are splenogastric, splenorenal and splenophrenic. This fact is through different to the rabbit because the gastro-splenic ligament is the only one described as present that the ligaments of the

spleen in equine and human in which our study in gazelle is in agreement with (12).

The splenic artery, in most studied gazelle samples, was branched inside the parenchyma of the spleen in gazelle. It was subdivided into multiple branches at the hilum. It is divided into three primary branches in contrast to the spleen of pig (13). The spleens of Gazelle was generally composed of two main parts; the stroma and the parenchyma. Actually, the spleens of other mammals have same principle structures in human and dog (14), in camel (15) in cat, sheep, cow and dog (16) and in rabbit (17).

Present microscopic findings showed that the capsule of spleen is composed of two layers, the outer one consists mainly of dense connective tissue; whereas the inner layer of the capsule consist of connective tissue fibers in addition to abundance of smooth muscle fibers interweaving among the connective tissue fibers. The white pulp in gazelle is demarcated by circumferential reticular fibers and dense lymphocyte populations that were divided it into two compartments, one surrounding the lymph follicles and the other forming a network at the PALS. These observation were in agreement with the finding of (18) in mice and (19) in man. They concluded that this reticular framework plays an essential role in lymphocyte homing and compartmentalization, which may also be true for the gazelle.

The red pulp was constructed mainly from cords of connective tissue extends and anastomose with each other in the parenchyma of the spleen. Small sinuses or sinusoids filled with blood extend among these cords and lined by flattened endothelial cells with relatively large spaces or slits between these cells. (9) stated that the red pulp found in Awasi sheep consist of sinusoid and pulp cord similar to the observation of (15). Other cells like lymphocytes, reticular cells, plasma cell, macrophages and megakaryocytes found in these sinusoids.

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### دراسة نسيجية-شكلية قياسية للطحال في الغزال المحلي (*Gazella subgutturosa*)

محمد هلال عبد مصلح و شاكر محمود مرهش

فرع التشريح والانسجة، كلية الطب البيطري، جامعة بغداد، العراق.

E-mail: [shakirmirhish@gmail.com](mailto:shakirmirhish@gmail.com)

#### الخلاصة

هدف البحث إلى دراسة الخصائص التشريحية والنسجية للطحال في الغزال المحلي. ، استعمل ٧ عينات من الطحال. كشفت الدراسة التشريحية أن الطحال في الغزال ذات لون بني داكن بيضوي الشكل يقع على السطح الجانبي الأيسر من الكرش في المساحة الممتدة بين الضلع الثامن والضلع الحادي عشر. حُسب الطول والعرض للطحال في الغزال وكان متوسط الطول والعرض  $7,94 \pm 0,1$  ،  $5,88 \pm 0,2$  سم على التوالي. تبين وجود ثلاثة أربطة تربط الطحال مع الاعضاء الأخرى وهي الرباط الطحالي المعدي، الرباط الطحالي الكلوي والرباط الطحالي الحجابي. دُرِس المدد الدموي للطحال باستعمال الأشعة السينية وطرق حقن الراتنج لوحظ أن الشريان الطحالي ينقسم إلى ثلاثة فروع ابتدائية، كل واحد من هذه الفروع تنقسم إلى فرعين ثانويين والتي تستمر في إعطاء الفروع الثلاثية التي تتوزع في متن الطحال. أظهرت هذه الدراسة أن محفظة طحال الغزال مكونة من طبقتين، الطبقة الخارجية تتكون أساساً من النسيج الضام الكثيف؛ في حين أن الطبقة الداخلية تتكون من ألياف النسيج الضام بالإضافة إلى وفرة من ألياف العضلات الملساء تتشابه بين ألياف النسيج الضام. وكان متوسط سمك المحفظة الكلي  $210,51 + 8,3$  ميكرومتر في حين كان سمك الطبقة العضلية الداخلية من المحفظة  $116,7 + 4,1$  ميكرومتر. كانت معظم الحويصلات مستديرة أو بيضاوية الشكل في الغزال و احتلت الحويصلات حوالي 9,6% من مساحة طحال الغزال. وأظهرت دراستنا أن متن الطحال يتكون من اللب الأبيض ممثلة بالعقيدات اللمفاوية مع منطقتها الهامشية والغمد اللمفاوي حول الشريان، واللب الأحمر ممثلة بالحبال الطحالية والجيبانيات. تبين أن معظم أقسام اللب الأبيض من الغزال ظهرت ذو مراكز جرثومية ضيقة أو غير محددة المعالم وكذلك المنطقة الهامشية. تم حساب أقطار الجربيات اللمفاوية، المراكز الجرثومية وسمك الغمد اللمفاوي حول الشريان في هذه الدراسة. وبلغت مساحة اللب الأبيض حوالي 9,6%. معظم مساحة الطحال. ظهر اللب الأحمر في طحال الغزال انه يتركب بشكل رئيس من حبال النسيج الضام والجيوب الصغيرة أو الجيبانيات الملينة بالدم ممتدة بين الحبال الطحالية ومبطنة بالخلايا البطانية المسطحة مع وجود مساحات كبيرة نسبياً أو شقوق بين هذه الخلايا. خلايا أخرى مثل الخلايا الليمفاوية، خلايا شبكية، خلايا البلازما، الضامة والخلية النواء وجدت في هذه الجيوب أو الجيبانيات. ظهرت الخلايا البلعمية كخلايا فاتحة كبيرة مع نواة بيضاوية فاتحة اللون، في حين أن الخلية النواء الضخمة ظهرت كبيرة ذات سيتوبلازم حامضي ونواة بيضوية ممطوطة غامقة.

الكلمات المفتاحية: غزال، طحال، اللب الاحمر، اللب الابيض، الغمد اللمفاوي حول الشريان.