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The greater omentum and similar serous formations of testis in male white rats

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Background: *The greater omentum of white rats appears, in basic morphological features (in miniature), to be homologous to the greater omentum of humans. We study of the greater omentum reaction to the catgut implant. After implantation of the catgut thread, it turned out that not only the greater omentum, but also serous formations similar to it, related to the testicles, are involved in the covering of the implant. The aim of the study was to study the general plan of the structure and the principles of morphometric analysis of serous formations of testis in white male rats.*

Materials and methods: *The experiment involved 15 white male rats of reproductive age, weighing from 284 to 334 grams.*

Results: *It has been established that each testicle of white rats has serous (peritoneal derivatives) formations of two types. One of them is a typical mesentery, with which each testicle is separately fixed to the posterior wall of the pelvic cavity, and the other formation is a free regrowth of a duplication of the serous membrane. It was called the epididymal omentum. According to the algorithm for studying the greater omentum in our previous works, it is noteworthy that the area of the greater omentum is noticeably inferior to the area of the epididymal omentums ($F = 0.239$; $p = 0.006$). So, if the average value of the area of the greater omentum is 2766.51 ± 388.12 mm^2, then the same indicator of the epididymal omentum reaches 4383.36 ± 793.56 mm^2, with their approximately the same thickness ($F = 1.35$; $p = 0.291$).*

Conclusions: *It has been established that the greater omentum has two, homeomorphic to it, derivatives of the peritoneum, associated with the epididymis, which were justifiably called epididymal omentums and were fully described in the literature for the first time.* (Folia Morphol 2023; 82, 4: 854–861)

Key words: greater omentum, epididymal omentum, vascular-fatty arcades, radial vascular-fatty tracts, serous-reticular membrane, adipocytes, aseptic inflammation, serous membrane

INTRODUCTION

Information on the features of the anatomical structure of the internal organs of white rats can be obtained from the works of many authors who are engaged in experimental modelling of various pathological conditions, and according to these authors, some organs of humans and white rats have more similarities than differences [12–14, 26, 29].

The greater omentum of white rats appears, in basic morphological features (in miniature), to be

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homologous to the greater omentum of humans [20, 27, 30].

The data obtained in previous works indicate that the greater omentum of white male rats can be in a latent form, being mostly located among the loops of the small intestine and in its usual state — located on the loops of the small and large intestine and consists of two component formations. They are represented by arcuate prostrate and anastomosing between themselves vascular-fatty arcades, which are linked by areas of the thinnest duplication of the serous membrane, called serous-reticular membranes. Each of them can be represented as a finely perforated thinnest duplication of the peritoneum. Its reticular structure can be distinguished relatively wide looped strands, surrounded by mesothelium, and narrow, variable in configuration membranes, which transversely connect them [18].

These data were obtained in order to conduct further experimental studies – the study of the greater omentum reaction to the catgut implant. After implantation of the catgut thread, it turned out that not only the greater omentum, but also serous formations similar to it, related to the testicles, are involved in the covering of the implant. It should be noted that these formations appear in the literature under such different names as: "gonadal depot of visceral white adipose tissue", "epididymal white adipose tissue", "gonadal fat", "epididymal fat", "epididymal fat pad" [1, 5, 9, 16, 17]. Gonadal white adipose tissue in female rats surrounds the uterus and ovaries and is called ovarian or parametrial white adipose tissue. In male rats, it's connected with the epididymis and with the testicle itself and is called epididymal white adipose tissue [8, 23]. According to the literature, the cytoarchitectonics of epididymal fat is represented by several types of cells, such as preadipocytes, mature adipocytes, immune and endothelial cells [3, 7]. But a sufficiently intelligible description of the structure of these formations, no matter what they are called, was not found in any source of literature. It forces us to conduct a thorough study in the same algorithm that we were guided by when studying the greater omentum, in determining the most accessible and indicative for them metric analysis of morphological dimensions, which will serve as control criteria in evaluating the results of planned experimental investigation.

The aim of the study was to study the general plan of the structure and the principles of morphometric analysis of serous formations of testis in white male rats.

MATERIALS AND METHODS

The experiment involved 15 white male rats of reproductive age, weighing from 284 to 334 grams. All animals were kept under standard conditions of the experimental biological clinic (vivarium) of the Poltava State Medical University, in accordance with the rules for keeping experimental animals established by the Directive 2010/63/EU of the European Parliament and of the Council, by Order of the Ministry of Education, Science, Youth and Sports of Ukraine No. 249 of 01.03.2012 "On Approval of the Procedure for Carrying out Experiments, Experiments on Animals by Scientific Institutions" and "General Ethical Principles of Animal Experiments", adopted by the Fifth National Congress on Bioethics (Kyiv, 2013), (Report No. 198 of 21.10.2021 from the meeting of the Commission on Biomedical Ethics of the Poltava State Medical University) [10, 21].

Vivisection was carried out in accordance with all the norms and requirements for conducting acute experiments on animals. The abdominal cavity was opened in all animals one-by-one (on a dissecting device in position of the animals on the back). It created a complete overview of the internal organs in their natural proportions [11].

Before further manipulations, at first, the entire content of the peritoneal cavity was subjected to gentle washing with warm 0.9% saline NaCl solution, and then irrigated from a syringe with 10% neutral formalin solution. Some total preparations of omentums were stained in a solution of haematoxylin and eosin and Van Gieson. Only after that overview photographs were taken with a digital camera directly in the body of a laboratory animal on laminated graph paper and matte glass.

At the same time, in such a straightened form of preparations, it was possible to carry out their metric measurements, which were carried out using the electronic calliper "Miol". Initially, the length and width of the omentums were measured. The length was measured from the point of the proximal-fixed side to the farthest point of its free-distal edge. The width was measured within its lateral dimensions. And if these two opposite sides are supplemented with two lateral lines, then the all omentum fits into a rectangle of a definite area. The area, of course, will be the derivative of two mutually perpendicular linear

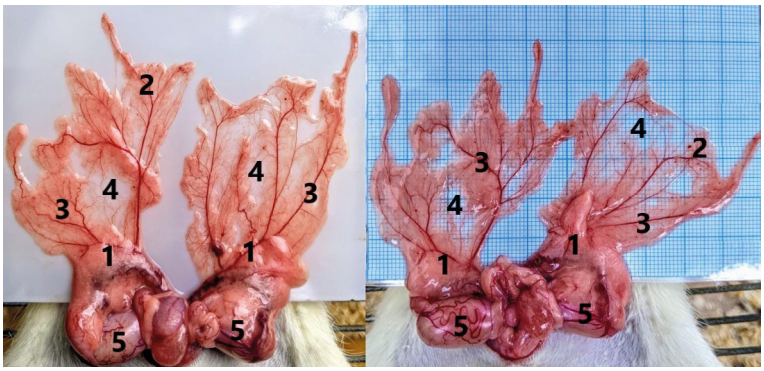

Figure 1. General view of the total preparation of the epididymal omentums of a sexually mature male rat; 1 — the base of the omentum; 2 — free edges of the omentum; 3 — radial vascular-fatty tracts; 4 — serous-reticular membranes; 5 — testicles.

distances. Finally, using the methods of variational statistics, we obtained the mean metric parameters of the epididymal omentum area and the greater omentum area of white rats. The second general morphometric parameter of the omentums was their thickness. But, due to the fact that it is variable over its entire area, this parameter can be obtained indirectly, based on its thickest irregularities. First, the thickness of the two slides was measured, then the fixed and free edges of the omentum were alternately placed on the first slide, covered with the second slide, and the thickness of the received "sandwiches" was measured. The desired index of the thickness of the two edges of the omentum was obtained by subtracting the known thickness of both slides from the total obtained value. Thus, the arithmetic mean of the two found indicators served as the general thickness of the epididymal omentum and the greater omentum of white rats. Only after that overview photographs were taken and it was proceeded to the direct study of the objects, followed by statistical processing of the obtained mathematical data.

Statistical analysis of the results was carried out on a personal computer using the Prism 5 (version 5/03) and Microsoft Excel 2010 software packages, descriptive statistics and statistical analysis methods. The description statistics are presented for the mean ± standard deviation. Quantitative values were presented in terms of median and interquartile (25%–75%) range (Q1–Q3).

The study was carried out in a Konus light microscope equipped with a Sigeta DCM-900 9.0MP digital microphotographic attachment with the Biorex 3 programme adapted for these studies (serial number 5604). Morphometric characteristics of the tissue structures of the corresponding preparations were obtained using a system for visual analysis of histological preparations, as well as using a Sigeta X 1 mm/100 Div. x0.01 mm object micrometer, the scale of which (equal to 1 mm, where the small division corresponds to 10 μm) was applied on the corresponding micrograph were obtained at an equivalent magnification.

RESULTS

With a sufficiently wide opening of the peritoneal cavity of animals and a comprehensive examination of its content, one can easily determine the location of the desired formations, which in intact animals are located in different positions. Thus, in some cases, they occupy a superficial location accessible for direct observation; in other animals, they are hidden (like the greater omentum) among the loops of the small intestine, and sometimes they can be found within the pelvic cavity and even immersed in scrotum [18]. But in all cases, they can be extracted together with the testicles, followed by the manufacture of visual total preparations, which were placed on thin frosted glass or laminated graph paper (Fig. 1).

It has been established that each testicle of white rats has serous (peritoneal derivatives) formations of two types. One of them is a typical mesentery, with which each testicle is separately fixed to the posterior wall of the pelvic cavity, and the other formation is a free regrowth of a duplication of the serous membrane. It was called the epididymal omentum (this name will be fully justified below). Actually, these serous formations belong to the sphere of our interests. Attention is drawn to the great diversity of their shape, which refers not only to individual differences, but also to bilateral asymmetry. But, along with this, there are fan-shaped forms, as well as resembling peculiar lobes or a petal, which has

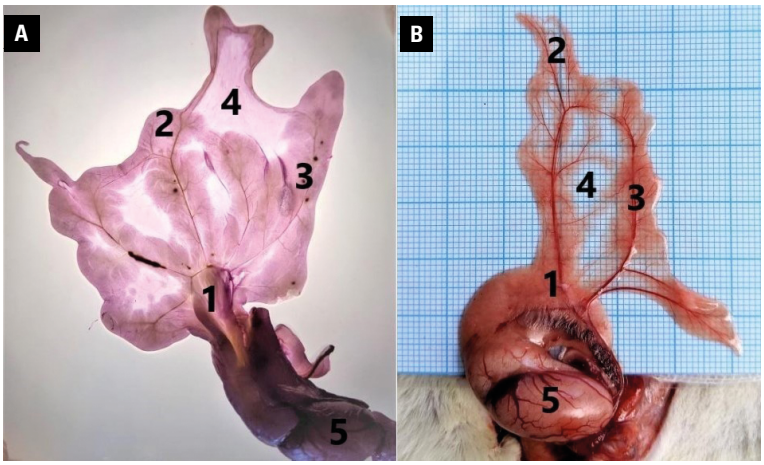

Figure 2. Various forms of epididymal omentums in male white rats. Total preparations; **A.** Stained with haematoxylin and eosin; **B.** Intact, unstained preparation; 1 — the base of the omentum; 2 — free edge of the omentum; 3 — radial vascular-fatty tracts; 4 — serous-reticular membranes; 5 — testicle.

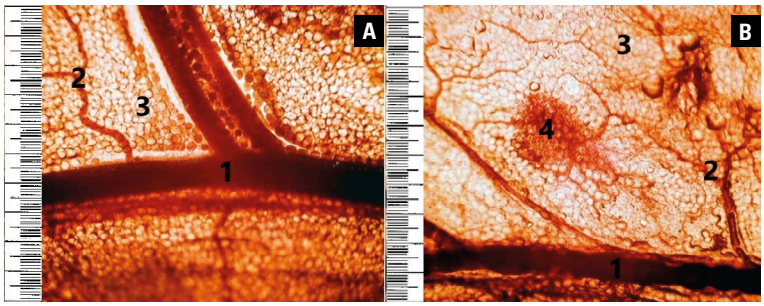

Figure 3. A, B. Areas of preparations of the epididymal omentum of a white male rat, totally stained with haematoxylin and eosin; 4× objective lens; small division of the metric scale: 10 microns; 1 — axial blood vessels; 2 — blood microvessels; 3 — fat cells; 4 — milky spot.

a narrow and short base, which starts from the head of the epididymis (Figs. 1, 2).

Starting from the head of the epididymis, blood vessels, which branch radially in the duplication of this serous lobe, penetrate through the narrow base of the epididymal omentum, which can be called a stem or pedicle. At the same time, in each radially oriented blood tract, there is an arterial vessel closely accompanied by a venous vessel (Fig. 3A). It should be noted that, in the greater omentum, the origins of the vascular-fatty arcades originate along the width of its base from the duodenum, greater curvature of the stomach and spleen.

It is quite remarkable that each radially oriented vascular tract occupies an axial position in the limbic adipose tissue, which has a lobular distribution. At the same time, along the length, they gradually become thinner to terminal microvessels. This picture exactly corresponds to the structural organization of the vascular-fatty arcades of the greater omentum, with the only difference being that anastomosing occurs between their terminal sections in the area of the free edge of the greater omentum [18]. But unlike the greater omentum in the serous lobes of the testicles, similar formations are not arcade, but radial in shape, which gives reason to call them radial vascular-fatty tracts. Basically, they are homeomorphic formations. This is also confirmed by the fact that in the fatty lobules of the radial vascular-fatty tracts of the serous lobes of the testicles, there are separate milk spots (Fig. 3B). However, compared to the greater omentum, they are much less common.

An expressive similarity between the structure of the greater omentum and the serous lobes of the rat testis lie in the presence of a reticular structure of intermediate zones between their radial vascular-fatty tracts, which were called serous-reticular membranes. They clearly coincide with each other in their architectonics (Fig. 2). In support of this, we present only some micrographs of the intermediate zones of the

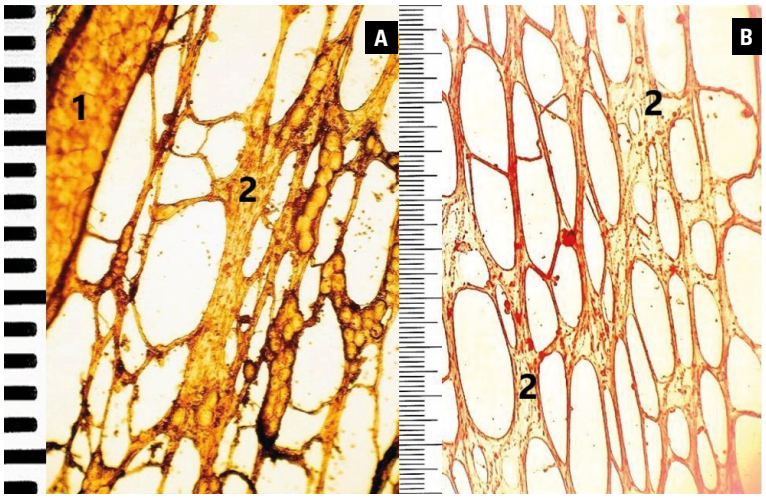

Figure 4. Areas of the serous intermediate zones of the epididymal omentum (**A**) and greater omentum (**B**) of a white male rat; **A.** 40× objective lens, stained with haematoxylin and eosin; **B.** 10× objective, Van Gieson staining; small division of the metric scale: 10 microns; 1 — tissue structures of the radial vascular-fatty tracts on the border with serous-reticular membranes; 2 — looped strands of serous-reticular membranes.

Table 1. Metric parameters of the epididymal omentum of white rats at the macroscopic level (n = 15)

	Min	Q1	Median	Q3	Max
Length [mm]	43.52	57.96	62.62	68.77	87.92
Width [mm]	52.88	63.94	69	75.14	90.01
Area [mm^2]	3222.43	3928.48	4348.74	4838.24	6245.84
Thickness [mm]	0.49	0.54	0.57	0.61	0.69
The lateral width of the radial vascular-fatty tracts of the epididymal omentum [mm]	2.7	3.65	3.81	4.2	5.02
The medial width of the radial vascular-fatty tracts of the epididymal omentum [mm]	3.56	5.03	5.22	6.16	7.08

Min, Max — minimal and maximal value; Q1 — first quartile; Q3 — third quartile

serous lobes of the testicles, which are comparable to those of the greater omentum (Fig. 4).

According to the algorithm for studying the greater omentum in our previous works, it has the following dimensions (on average, length 43.61 ± 3.50 mm; width 63.22 ± 5.69 mm; area 2766.51 ± 388.12 mm^2; thickness 0.52 ± 0.07 mm), its vascular-fatty arcades had the following width (duodenal arcade 3.58 ± ± 0.99 mm; gastric arcade 2.28 ± 0.35 mm and splenic arcade 3.82 ± 1.16 mm). A morphometric analysis of the epididymal omentum was carried out. It was found that they differ in great variability in their size, that is, in the area of contact with the peritoneal fluid, in which they are located in a rather arbitrary arrangement and have the following dimensions (on average, length 63.36 ± 9.43 mm; width 69.54 ± 9.77 mm; area 4383.36 ± 793.56 mm^2; thickness 0.58 ± 0.06 mm), the width of the radial vascular-fatty tracts of the epididymal omentum was also measured (lateral tract 3.92 ± 0.52 mm and medial tract 5.59 ± 0.99 mm)

Table 1 shows the main numerical data reflecting the metric characteristic of one of the two epididymal omentums. In Table 2 is presents metric parameters of the greater omentum.

Despite the relativity of these metric parameters, they make it possible to judge the dimensional relationships between the serous formations and the greater omentum. First of all, it is noteworthy that, according to the obtained planimetric data, the area of the greater omentum is noticeably inferior to the area of the epididymal omentums ($F = 0.239$; $p = 0.006$). So, if the average value of the area of the greater omentum is 2766.51 ± 388.12 mm^2, then the same indicator of the epididymal omentum reaches 4383.36 ± 793.56 mm^2, with their approximately the same thickness ($F = 1.35$; $p = 0.291$).

The area of epididymal omentums has a large individual variation ranging from 3222.43 to 6245.84 mm^2. Noteworthy is the fact that this range of individual variability depends mainly on the length ($r = 0.669$;

Table 2. Metric parameters of the greater omentum of white rats at the macroscopic level (n = 15)

	Min	Q1	Median	Q3	Max
Length [mm]	35.65	41.61	43.27	45.62	48.68
Width [mm]	50.64	59.96	63.55	66.49	72.60
Area [mm^2]	1805.31	2544.03	2804.89	2988.99	3354.98
Thickness [mm]	0.40	0.48	0.52	0.56	0.69
The duodenal width of the vascular-fatty arcades of the greater omentum [mm]	2.03	3.01	3.45	4.15	5.88
The gastric width of the vascular-fatty arcades of the greater omentum [mm]	1.85	2.07	2.19	2.48	3.01
The splenic width of the vascular-fatty arcades of the greater omentum [mm]	1.97	3.16	3.99	4.49	5.80

Min, Max — minimal and maximal value; Q1 — first quartile; Q3 — third quartile

$p = 0.006$), while the area of the greater omentum varies individually mainly due to its width ($r = 0.873$; $p < 0.0001$).

Additional morphometric information was obtained by measuring the width of the radial vascular--fatty tracts of the epididymal omentum (Table 1). They differ from the vascular-fatty arcades of the greater omentum only in shape, but not in their internal structure, except that in the epididymal omentums, they are, according to average statistics, somewhat wider ($F = 28.08$; $p < 0.0001$) due to a greater deposition of adipose tissue in them on the sides of the axial blood vessels.

DISCUSSION

According to the world literature, visceral white adipose tissue plays an important role in the accumulation and release of energy, maintaining homeostasis, thermoregulation, secretion of adipokines in order to regulate metabolism, immune reactions, and maintaining the body's energy balance. Visceral adipocytes surround vital organs and are contained in the gonadal, perirenal, retroperitoneal, omentum, and pericardial depots. According to research by Bagchi and MacDougald [1] epididymal white adipose tissue is connected to the epididymis and blood vessels, which is also confirmed by our research. In turn, there may be several visible blood vessels and they are oriented and branched radially, occupying an axial position — that is why we call them radial vascular-fatty tracts, about which there are no data in the literature.

In the conducted literature search, no attention is paid to determining the shape of epididymal serous formations of the testicles. In our work, we drew attention to the variety of forms and bilateral asymmetry of epididymal omentums, which, starting with a narrow and short leg and can have a petal-like or fan-like shape.

In the process of research, we didn't find comparative literary data of the greater omentum with epididymal fat; therefore, we made an attempt to compare these formations and as a result of the research, the undeniable similarity of the structural elements of their morphological structure was revealed. Based on the obtained results, we think it is more correct than the existing terms in the literature to call these serous formations of the testicles epididymal omentums.

During the anatomical study of epididymal fat, some authors distinguish the proximal and distal part of the epididymal fat pad [15, 17].

While studying the external structure of the epididymal omentums, a short base that starts from the head of the epididymis and a free edge where it ends were identified. This fact was investigated and confirmed by Berry et al. [2, 22].

We also single out the mesentery, which fixes the testicle directly to the back wall of the pelvic cavity, and the actual free growth of the duplication of the serous membrane with adipose tissue.

During the morphometric analysis, it was established that the area of the epididymal omentum exceeds the area of the greater omentum — mainly due to the length, which is confirmed by the studies of other authors [5, 24], who recognise epididymal fat as the largest representative of white visceral fat in rodents.

According to Cleary et al. [7] and, Billon and Dani [3], epididymal fat is composed of several cell types, including mature adipocytes of various sizes, immune cells, endothelial cells, and preadipocytes. At the microscopic examination, milky spots, endothelial cells, and adipocytes were found, but we did not differentiate the maturity of adipocytes or their precursors. The number of milky spots in the epididymal omentum, according to the observation, is significantly less than

in the greater omentum, which coincides with the data of the literature about milky spots of the greater omentum and the gonadal fat [19, 25, 28].

At present, the function of epididymal fat is not clearly identified. According to Chu et al. [6], surgical removal of epididymal fat causes cessation of spermatogenesis in the testis. It is suggested that secretory molecules derived from epididymal fat, such as leptin, resistin, and adiponectin, may influence the regulation of testicular function. A group of other authors believes that gonadal white adipose tissue may regulate gametogenesis by modulating neuroendocrine signalling [16, 17, 31].

We, in turn, without delving into molecular research, noted the active participation of the epididymal omentum in acute inflammatory processes of the abdominal cavity, namely, the prevention of the spread of the peritoneal inflammation (peritonitis, both septic and aseptic), which is the perspective of our further research.

Also, the epididymal omentum deserves attention, especially when conducting experimental studies on laboratory animals, as it is absent in humans, which was also noted by some authors [4].

Thus, when comparing the anatomical structure of the structural components of the greater omentum with the epididymal omentum, we consider it appropriate to call the vascular ways of the greater omentum — vascular-fatty arcades, and the epididymal omentum — radial vascular-fatty tracts; intermediate avascular areas of omentums — serous-reticulate membranes, which are similar in their structure. This is also confirmed by the fact that in the fatty lobes of the radial vascular-fatty tracts of the testicles there are individual milky spots located along the blood vessels [18].

Therefore, the conducted brief comparative analysis between the structure of the greater omentum and the serous formations of the testicles of white rats shows that, due to their combination of similar tissue components, they can be considered as homologous derivatives of the peritoneum. Based on this thesis, we consider it is more correct than the existing terms in the literature to call these serous testicular formations epididymal omentums, as mentioned above, since they begin with short stems (or pedicles) from the head of the epididymis of the corresponding testicle.

CONCLUSIONS

We established that the greater omentum has two, homeomorphic to it, derivatives of the peritoneum, associated with the epididymis, which were justifiably called epididymal omentums and were fully described in the literature for the first time.

Thus, in the peritoneal cavity of white male rats, in contrast to humans, according to our data, there are not one but three omentums. Because, according to the structure and topology in the peritoneal cavity, the small and large omentums cannot be considered identical formations, since the small one belongs to the category of ligaments.

Conflict of interest: None declared

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