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Research Article

Comparative Analysis of the Human and Pig Vitreous Body Microsurgical Anatomy

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Abstract

Introduction: Microsurgical anatomy studies the structure and topography of small anatomical structures in relation to the requirements of microsurgery. The main methods of studying microsurgical anatomy of tissues are macroscopic dissection and the preparation of histotopograms [1]. Until now, the transparent vitreous body has not been considered as an organ that can be studied using the main methods of microsurgical anatomy. Since 2010, the S.N. Fedorov National Medical Research Center for Microsurgery of the Eye has been conducting research on microsurgical anatomy using the method of macro-microscopic dissection with the use of Vitreocontrast suspension. Since 2010, the S.N. Fedorov Federal State Institution "Microsurgery of the Eye" has been conducting research on microsurgical anatomy using the method of macroscopic dissection with the use of Vitreocontrast suspension as a contrasting agent. By using the injection method and the method of layer-by-layer contrasting during macroscopic dissection, it became possible to isolate the intravitreal structures for subsequent histological examination. These experimental studies were conducted on autopsied eyeballs from cadaveric donors. Other anatomical models of the vitreous body are required for the process of teaching microsurgical anatomy research methods.

Purpose: To compare the microsurgical anatomy of the vitreous body of human and a pig eyes.

Materials and Methods: In this work, a comparison was made of the microsurgical anatomy of the vitreous body of human and animal (pig) eyeballs. During the work, 20 autopsied eyes of cadaveric donors and 20 pig eyeballs were examined in a simulated operating room, wet lab.

Results: A comparative analysis of the results of a study of the microsurgical anatomy of the vitreous body, conducted using a similar method, including the method of macro-microscopic dissection with isolated isolation of the structures of the vitreous body and subsequent examination using light microscopy, revealed a similar anatomical and histological structure of the vitreous body in the pig and human eyes.

Conclusion: The results demonstrate a sufficient similarity in the anatomical structure of the vitreous body in pigs and humans, and therefore, the possibility of using pig eyes as an anatomical model for studying the microsurgical anatomy of the vitreous body and practicing the technique of its macroscopic dissection without the need to use cadaveric donor eyes. The technique demonstrates high visibility, reproducibility, and can be adapted for educational purposes within the framework of training courses in anatomy, histology, and ophthalmology.

Keywords: *Vitreous Body; Microsurgical Anatomy; Dissection; Training; Wet Lab*

Introduction

The study of eyeball dissection techniques is a crucial step in the training of future ophthalmic surgeons. Various methods are used to study the microsurgical anatomy of tissues, such as macroscopic-microscopic dissection, the histotopographic method, the microinjection method, and the layer-by-layer dissection method [1]. The vitreous body (VB) is a transparent gel-like structure consisting of 99% water. Dissecting this anatomical structure of the eyeball is a technically challenging task. The first studies of the internal structure of the VB using dyes to visualize intravitreal structures were conducted by J.G.F. Worst and Z.A. Makhacheva [2]. Histological examination of the VB presents certain difficulties, as it is often performed without isolating anatomical structures, which hinders the correct interpretation of results. Kislitsyna N.M. and co-authors proposed a method for the macroscopic and microscopic examination of human VB called Step by Step [2], which used vitreocontrastography technology to isolate any anatomical structure of the VB and perform a layer-by-layer analysis of the cortical layers, preserving their integrity and shape after all stages of histological processing.

However, the process of learning microsurgical anatomy techniques requires different vitreous body (VB) anatomical models. Currently, the study of the anatomy of the eyeball and the development of microsurgical skills by ophthalmologists take place in specially equipped laboratories (Wet Labs). Enucleated (cadaveric) animal eyes are used for this purpose [3].

Purpose of the Study

The purpose of this study was to develop a method for investigating the microsurgical anatomy of the cornea using a porcine eye model in the simulated operating room (Wet Lab).

Materials and Methods

The porcine eyes were enucleated from 8- to 9-month-old Hypor Kanto pigs. The material was delivered within 12 to 18 hours. The material was not frozen.

The study included 20 cadaveric donor eyeballs with removed corneoscleral discs; the donors' ages ranged from 47 to 61 years, with 12 males and 8 females; the cause of death was acute cardiovascular failure.

The eyeballs underwent quality control for corneal transplantation at the Eye Tissue Bank of the MNTK "MG" Moscow, in accordance with the medical technology authorization FS No. 2010/243 dated June 24, 2010, "Algorithm for the procurement of human cadaveric corneas for transplantation," and the Institution's Licenses No. 99-01-005317 dated April 30, 2008, issued by the Federal State Service for Health Supervision, and No. FS-99-01-008251 dated February 18, 2013, for the medical activity of harvesting and processing human cadaveric organs and tissues for transplantation. The dissection of the eyeballs was performed within the first 12 hours after their arrival at the Eye Bank.

The study was conducted in a simulated operating room (Wet Lab) under the magnification of surgical microscopes manufactured by Zeiss, Opton, and Leica, equipped with photo and video recording devices. A set of microsurgical instruments and stands for fixing the eyeball were used to perform macroscopic and microscopic dissection.

To prepare the material for examination by light microscopy, an Endokit, a biopsy bag, and a biopsy cassette were used.

The preparation of the VB specimen was performed using a previously developed original method for the macroscopic and microscopic examination of human VB specimens [4].

To visualize the structures and layers of the VB an original method (vitreocontrastography) was used based on preliminary contrast enhancement using layer-by-layer and injection methods (Russian Federation Patent No. 2441629). During the macromicroscopic

examination, the "Vitreocontrast" suspension was used as the contrast agent. This contrast agent (Technical Specification No. 9398-017-29039336-2009) is an ultradisperse suspension based on a non-toxic inorganic barium sulfate salt in an isotonic solution with an osmolarity of 300 - 350 mOsm. Barium sulfate is present as white crystals with a molecular weight of 233.43 g/mol, a particle size in suspension of less than 5 microns, and a density of 4.4 g/cm^3. Each milliliter of sterile solution contains 140 mg of barium sulfate (dry weight).

Stages of macroscopic and microscopic dissection:

1. Assessment of anatomical and topographical changes in the vitreoretinal interface (using the ZOST induction method).
2. Assessment of the topographical anatomy of intravitreal structures (channels, pouches, cisterns).
3. Assessment of the topographic anatomy of the anterior cortical layers and the vitreolenticular interface.

During the macroscopic examination, vitreocontrastometry may be performed to visualize the boundaries of intravitreal structures and accurately measure the dimensions and topography of the enhanced vitreous structures and layers.

Microscopic (histological) examination of isolated VB structures and layers:

1. Isolated dissection of VB structures and layers.
2. Fixation using a proprietary method with a special substrate.
3. Evaluation of microscopic changes using the appropriate method (light or electron microscopy, immunohistochemical examination).

Results

The steps involved in dissecting porcine eyes and cadaveric donor eyes followed the procedure described earlier, with the anatomical structures being separated in virtually the same manner (Figure 1-4).

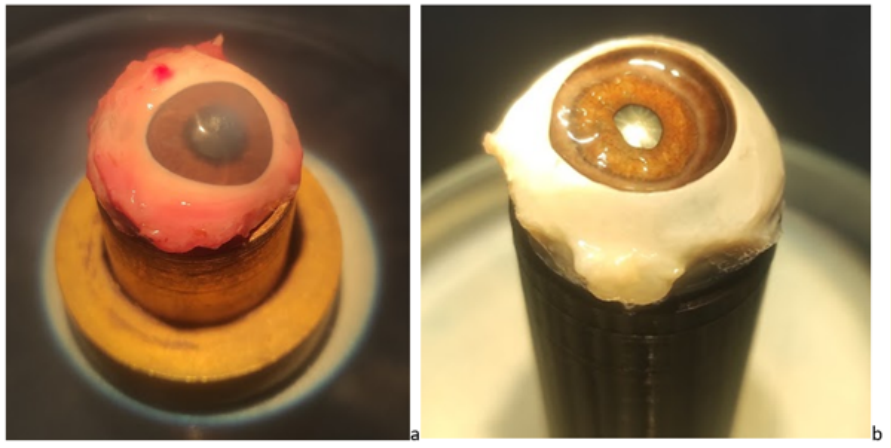

Figure 1: *Porcine and cadaveric donor eyeballs fixed on holders: a) Porcine eyeball. b) Human cadaveric donor eyeball.*

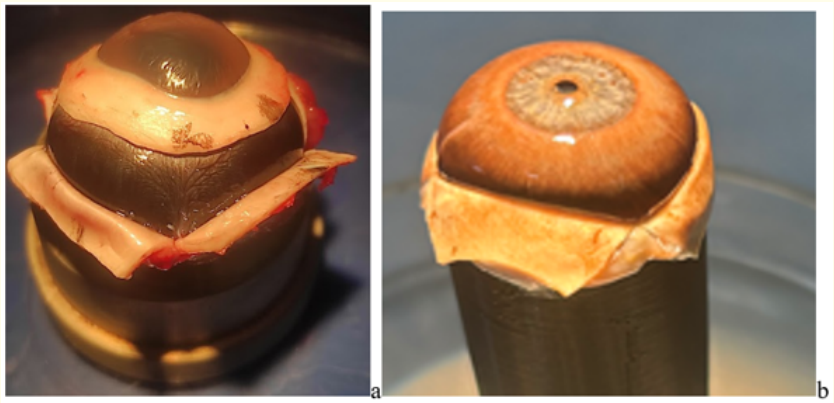

Figure 2: *Stages of macro-microscopic dissection. Scleral flaps have been created. The sclera is separated from the choroid. The choroid is visualized: a) Porcine eyeball. b) Human cadaveric donor eyeball.*

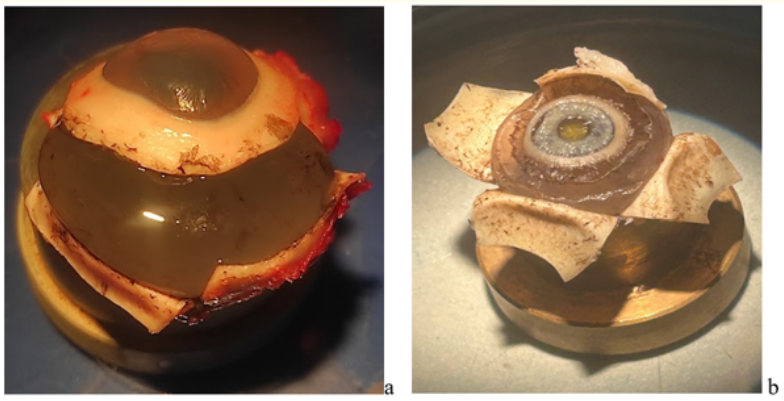

Figure 3: *Stages of macro-microscopic dissection. The choroid is separated using forceps. The retina overlying the vitreous body is visualized: a) Porcine eyeball. b) Human eyeball.*

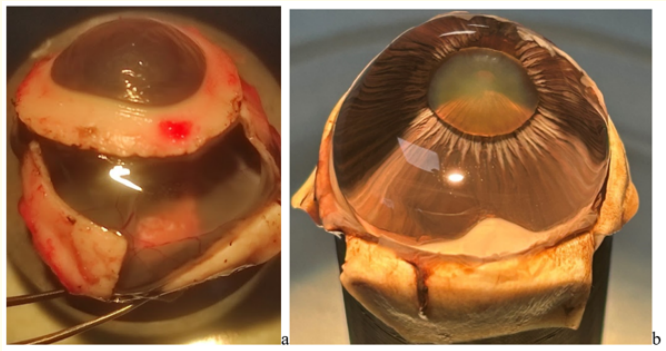

Figure 4: *Stages of macro-microscopic dissection. The retina is separated from the surface of the vitreous body. The transparent vitreous body is visualized: a) Porcine eyeball. b) Human cadaveric donor eyeball.*

After the separation of the eye membranes, the VB did not lose the volume and retained its shape. To preserve the anatomical and topographical relationships, the anterior segment and the posterior pole of the eye were not separated at this stage (with the exception of cadaveric donor eyeballs after the cornea transplant recovery).

Vitreocontrast suspension was used to enhance vitreous structures. A 30G needle was used to inject the stain agent into the vitreous structures. The cisterns were injected with 0.2 mL of contrast suspension; the needle was inserted 4 mm from the limbus. The degree of structural preservation was assessed, and the dimensions of the cisterns and the extent of ST destruction were measured. To contrast the retrociliary cisterns, the needle was inserted at an angle of approximately 15° to a depth of 3-4 mm. To contrast the equatorial cisterns, the needle was inserted at an angle of approximately 30° to a depth of 3-4 mm. Access to the optic-ciliary canal was achieved retrograde (from the optic nerve head).

During macroscopic and microscopic dissection of the porcine eye, sac-like cavities (cisterns) were observed that were similar in topography, location, and shape to the cisterns previously identified and described in the eyes of cadaveric donors (Figure 5a and 5b) [2].

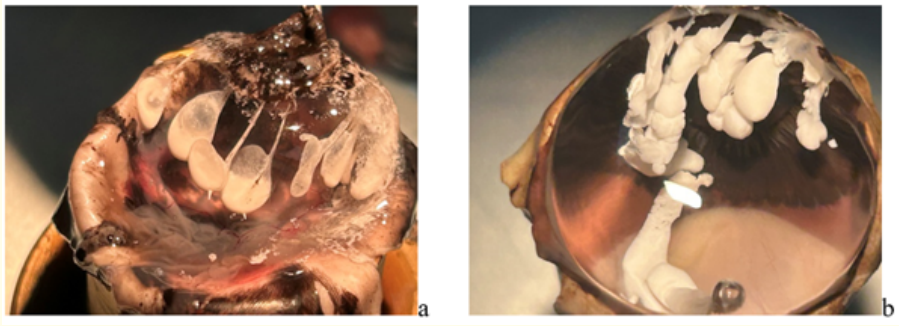

Figure 5: *Injection-based method of contrast enhancement of vitreous structures. Retro-ciliary cisterns are contrasted using the "Vitreocontrast" suspension: a) Porcine eyeball. b) Human eyeball.*

Using a direct ophthalmic caliper, the dimensions (length and width) of the chambers were measured (vitreocontrastometry), and the shape and integrity of the walls of the intravitreal structures were assessed (Figure 6a and 6b).

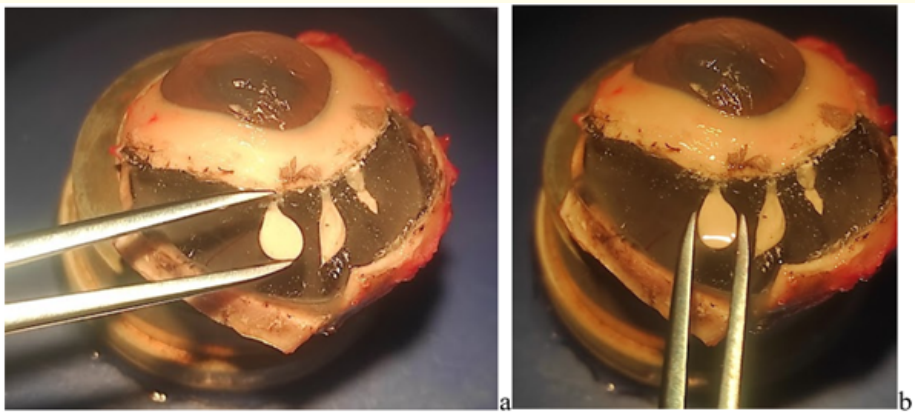

Figure 6: *Stages of macro-microscopic dissection of the porcine eyeball. Measurement of cistern dimensions after contrast enhancement: a) Length of the retro-ciliary cistern: 5 mm. b) Width of the retro-ciliary cistern: 3 mm.*

Next, we performed the dissection (isolated extraction) of the intravitreal structures; to do this, we incised the cortical layers of the VB and used microsurgical forceps and scissors to separate the stained cisterns.

To fix the vitreous samples, individual structures were fixed and placed into a biopsy bag, which was then stored in a biopsy cassette. Afterward, the isolated specimens were immersed in formalin and transported to the laboratory for standard processing [2].

To prepare samples for subsequent light microscopy, the isolated endo-vitreal structures were fixed on a Bio Optica adhesive-metric plate, measured, transferred to an Endokit substrate (Figure 7), which was placed in a biopsy bag, and then into a biopsy cassette for further examination. A histological examination of the intravitreal structures (cisterns) was performed, followed by interpretation of the results.

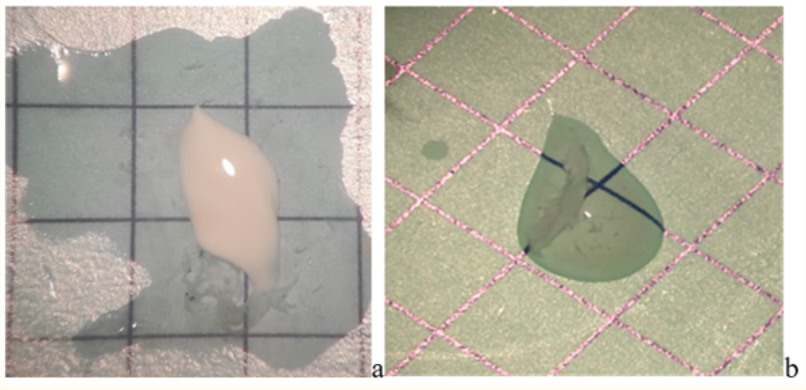

Figure 7: *Photograph of the isolated vitreous cistern. The isolated vitreous cistern mounted on a Bio Optica adhesive-metric plate (metric grid size 3 × 3 mm): a) Retro-ciliary vitreous cistern of the porcine eye. b) Retro-ciliary vitreous cistern of the human eye.*

For histological examination, vitreous structures were fixed in a 10% solution of neutral formalin, rinsed with running water, dehydrated in a series of increasing alcohol concentrations, and embedded in paraffin; a series of histological sections was prepared and stained with hematoxylin and eosin. Following slide preparation, microscopic examination was performed using a Leica DM LB2 microscope at 200× magnification, followed by photography (Figure 8).

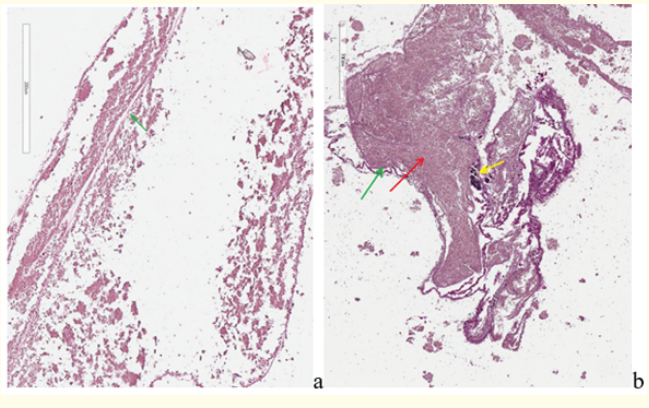

Figure 8: *Histological specimens of vitreous cisterns: a) Histological specimen of a porcine vitreous cistern (11655, ×20, slide 3). Two cisterns with a shared wall (indicated by the green arrow). b) Histological specimen of a human eye cistern at ×200 magnification (11534, ×40, slide 2). The green arrow indicates the wall of the vitreous cistern, the red arrow — "Vitreocontrast" dye, and the yellow arrow — the retinal pigment epithelium.*

Discussion

The study of the vitreous microsurgical anatomy within the fields of normal and pathological human anatomy, histology, and ophthalmology has received undeservedly little attention.

Microsurgical anatomy examines the structure and topography of small anatomical structures as they relate to the needs of microsurgery [1]. Until now, the transparent gel-like vitreous has hardly been considered an organ to which the basic methods of microsurgical anatomy apply.

Microsurgical anatomy combines two principles: the use of macroscopic and microscopic fields of view corresponding to the range of the surgical microscope, and the topographic principle of studying the structure and location of anatomical structures.

The methodological basis for the study of microsurgical anatomy consists of a set of techniques, in which macroscopic and microscopic dissection as well as the histotopographic method play a leading role.

In the classical form, macroscopic-microscopic dissection is a stereomorphological dissection method developed by Academician V.P. Vorobyov. This dissection was performed using microsurgical instruments with carefully selected lighting direction to provide data on the macromicroscopic structure of the blood supply to peripheral nerve plexuses. In addition to macroscopic-microscopic dissection, several techniques were used to study the structure and topography of anatomical elements within the range of optical magnification. These include microinjection and layer-by-layer dissection. The second method for studying microsurgical anatomy is histotopographic. A histotopogram refers to a stained histological section of an anatomical structure or organ. General histological staining methods—such as hematoxylin and eosin, Van Gieson: however, special staining methods may also be used depending on the object and research objectives.

One of the most challenging tasks is the dissection of the vitreous body, which, in its unfixed state, is a transparent, colorless, gelatinous mass filling the cavity of the eyeball [5]. Among the most significant works on the internal structure of the vitreous humor are the studies by Worst J.G.F. and Makhacheva Z.A., who first used dyes to visualize the vitreous humor [6]. To study VB microsurgical anatomy, macroscopic and microscopic dissection, the histotopographic method, the microinjection method, and the layer-by-layer dissection method were employed [1].

Histological examination of the vitreous body (VB) presents particular challenges, as it is often performed indiscriminately without identifying anatomical structures. The lack of specificity makes it difficult to interpret the results correctly [2,7].

Since 2010, the S.N. Fedorov Federal State Budgetary Institution National Medical Research Center “Eye Microsurgery” has been conducting research on microsurgical anatomy using the macromicroscopic dissection method with Vitreocontrast suspension as a contrast agent. Using the injection method and the layer-by-layer contrast method, it has become possible to isolate intravitreal structures for subsequent histological examination. These experimental studies are conducted on autopsied eyeballs from cadaveric donors.

Kislitsyna N.M. and co-authors proposed a Step by Step method [2] using vitreocontrastography imaging technology. This approach allows for the isolated enhancement of any vitreous anatomical structure including each cortical separately, while preserving their integrity and shape after all stages of histological processing.

However, the process of learning microsurgical anatomy techniques requires different vitreous anatomical models. Learning how to dissect the eyeball is one of the mandatory stages in the training of future ophthalmic surgeons[8]. The study of the vitreous body (VB) anatomy and the practical development of microsurgical skills take place in specially equipped laboratories—wet labs.

Animal eyes are most commonly used as models for practicing microsurgical procedures; specifically, the eyes of goats, sheep, rabbits, and pigs are used [3]. One of the eyes most similar in microsurgical anatomy to the human eye is a porcine eye, which has a pupil, a macular region, and so on [9]. Pig eyeballs have been used to develop microsurgical skills during training in the stages of ophthalmic surgery since the mid-20th century [3]. The pig eye is extremely convenient for educational purposes as methods for modelling various pathologies are well known, for example, the artificial creation of cataracts using microwave radiation [10].

To date, no methodology for teaching and practicing the macroscopic and microscopic dissection of porcine eyes in a wet lab setting - specifically for studying the vitreous microsurgical anatomy - has been used or described in the literature. The Step by Step method [2] served as the prototype for our algorithm for dissecting porcine vitreous body (VB). In the course of a comparative analysis of the results of a study on the microsurgical anatomy of the vitreous body (VB), conducted using a uniform methodology including macro-microscopic dissection with isolated extraction of VB structures and subsequent light microscopy, a similar anatomical and histological structure of the VB was identified in both the pig and human eyes.

Conclusion

The results obtained demonstrate the potential for using porcine eyes as an anatomical model for studying the microsurgical anatomy of the VB and for practicing macroscopic and microscopic dissection techniques. The method offers high clarity and reproducibility and can be adapted for educational purposes within training courses on anatomy, histology, and ophthalmology.

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