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PATHOMORPHOLOGICAL CHARACTERIZATION OF LENS CAPSULE TISSUES IN ANIMALS AFTER PREVENTION OF SECONDARY CATARACTS: AN EXPERIMENTAL STUDY

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Abstract

One of the current problems of veterinary science is the treatment and prevention of pathologies that reduce the working and productive qualities of especially valuable animals. In veterinary ophthalmology, it remains an important goal to find ways to prevent secondary cataract after ultrasound phacoemulsification. Different methods are used for this purpose, but the questions about pathomorphosis of secondary cataract and scientific basis of effective methods of its prevention remain open. In the present work morphologic changes in the lens capsule of rabbits 2 and 6 months after phacoemulsification under different methods of secondary cataract prophylaxis were identified for the first time. On the basis of clinical picture together with morphological changes the most effective method of prophylaxis - capsulorhexis of the posterior lens capsule - was established. The aim of the work was to study the features of morphological changes occurring in the lens capsule in rabbits after phacoemulsification combined with intraoperative techniques of secondary cataract prophylaxis. The study was carried out in 2020-2021 at the Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology named and at Dr. A.G. Shilkin Veterinary Ophthalmology Center on 18 crossbred rabbits (*Oryctolagus cuniculus domesticus*) of both sexes, aged 4 months, without ophthalmic pathologies. The animals were divided into three groups (6 animals in each group). Ultrasound phacoemulsification was performed in all groups according to the generally accepted technique. Animals of group 1 were implanted with intraocular lens (AquaFree Y-PL, hydrophobic, Overal Dia 13.0 mm, Rumex, USA), group 2 — with intracapsular ring (polymer ophthalmic intracapsular ring for lens bag spreading and lens capsule tensioning, KPV-"ETP-MG" ring No. 3, 13.0 mm, Federal State University Cross-Sectoral Research and Technology Centre, Acad. Fedorov Eye Microsurgery Rosmedtechnologiya-ETP-MG, Russia). In group 3 posterior capsulorhexis was performed. In the postoperative period, the rabbits received general (enrofloxacin, 5 mg/kg body weight, 2 times per day; ketoprofen, 3 mg/kg body weight, 1 time per day for 3 days) and topical treatment (atropine for 10 days; moxifloxacin, eye drops for 14 days; dexamethasone + neomycin + polymyxin B sulfate for 14 days; nepafenac for 28 days). The animals were under continuous monitoring, during which ophthalmologic examination and photographic fixation of the eye condition were performed. Signs of secondary cataract were identified by using slit-lamp biomicroscopy, the state of the ocular fundus was examined by ophthalmoscopy, and the transparency of the capsule was determined. The rabbits were subjected to drug euthanasia 2 and 6 months after the surgery, by 2 and 4 animals, respectively. Eyeballs were enucleated and placed in 10% neutralized formalin solution. For macroscopic evaluation of the state of the lens capsule, a slice was made in the area of the serrated edge of ora serrata with retinal capture. Most of the vitreous body was aspirated. After photography, a sagittal section of the eye was performed and subjected to histologic examination. It was found that within 2 months after performing the above techniques, proliferation of lens epithelium resumes in the capsule, lens fibers are formed (mainly in the area of the equator of the capsular bag), and adhesions with the iris may develop. Within 6 months, the changes progress, however, to a different degree depending on the technique used. It is shown that capsulorhexis of the

posterior lens capsule is the most effective technique for secondary cataract prophylaxis due to a limited impact on tissues in general, absence of alien objects after the surgery and, therefore, the least proliferation of pathological lens tissues with preservation of optical transparency of the central region of the capsular bag.

Keywords: lens, cataract, posterior capsulorhexis, capsule opacification, secondary cataract, phacoemulsification, secondary cataract prevention

The search for methods of treating animals with cataracts is an urgent problem in veterinary medicine, in particular in service dog breeding and sports horse breeding. The prevalence of lens pathologies in horses, especially thoroughbreds, is due to a significant incidence of uveitis (inflammation of the choroid); cataracts are recorded in 46-53% of all ophthalmopathies. Among lens pathologies in dogs, cataracts account for 90% [1]. This is one of the most common causes of decreased visual function in animals, leading to a decrease in the performance of dogs and their premature culling. An effective method for treating animals with cataracts is phacoemulsification which results in positive outcomeы in most cases. However, a common complication after surgery is secondary cataract which leads to a decrease in visual function [2] and, according to various sources, develops within 1-6 months after the intervention in 62% [3] and even in 100% of cases [4].

In human medicine, YAG laser discission successfully correct this condition, removing the optically opaque region of the capsule [5]. However, in veterinary medicine this technique is ineffective due to numerous complications. Thereof, methods of intraoperative prevention of secondary cataracts acquire particular importance [6, 7]. Among surgical techniques combined with phacoemulsification [8, 9], intraocular lens implantation has shown effectiveness in veterinary practice [10, 11]. There is limited information about the positive effects of mechanical polishing of the lens capsule and implantation of an intracapsular ring [12-15], and about capsulorhexis of the posterior lens capsule [16-21].

Discussing the pathogenesis of secondary cataracts, some authors [22, 23] describe the mechanism of fibrous metaplasia of lens epithelial cells. In their opinion, the cells of the lens epithelium in patients with cataracts underwent metaplasia with the formation of so-called plaques, consisting of connective tissue and ectopic basement membrane formed by epithelial cells. M.E. Bernays et al. [24] identified this condition in 7 of 25 cases studied. Despite the data obtained, the metaplasia of lens cells into fibroblasts followed by connective tissue formation is questionable. In addition, there has not yet been a comparative study of the changes, occurring in tissues after phacoemulsification combined with intraoperative techniques to prevent secondary cataracts. Rabbits are similar to horses and dogs in terms of inflammation and exudation types and can be used to develop methods for preventing secondary cataracts [25, 26].

In this paper, for the first time, morphological changes were identified in the lens capsule of rabbits 2 and 6 months after phacoemulsification with various methods of preventing secondary cataracts. Based on the clinical picture and morphological changes, the capsulorhexis of the lens posterior capsule has been established as the most effective method to prevent secondary cataracts.

The purpose of the work is to study morphological changes that occur in the lens capsule in rabbits after phacoemulsification combined with intraoperative manipulations for the prevention of secondary cataracts.

Materials and methods. Rabbits (*Oryctolagus cuniculus domesticus*) without ophthalmological pathology aged 4 months (n = 18, mixed breeds of both sexes) were used in the experiment (Scryabin Moscow State Acafemy of Veterinety Medicine and Biotechnology, the Center for Veterinary Ophthalmology of Dr. A.G. Shilkin, 2020-2021).

The rabbits were assigned to three groups, 6 animals in each. In all groups, ultrasound phacoemulsification was performed according to the generally accepted technique. Animals of group 1 were implanted with an intraocular lens (AquaFree Y-PL, hydrophobic, Overal Dia 13.0 mm, Rumex, USA), animals of group 2 were implanted with an intracapsular ring (a polymer ophthalmic intracapsular ring for straightening the lens bag and tensioning its capsule, CPV-ETP-MG ring No. 3, 13.0 mm, Fedorov MNTK Eye Microsurgery, Rosmedtekhnologii-ETP MG, Russia). In group 3, posterior capsulorhexis was performed. In the postoperative period, the rabbits received enrofloxacin (5 mg/kg bodyweight 2 times a day), ketoprofen, 3 mg/kg BW, 1 time a day for 3 days) and local application of atropine for 10 days, moxifloxacin eye drops for 14 days, dexamethasone + neomycin + polymyxin B sulfate for 14 days, and nepafenac for 28 days.

The animals were under observation during which an ophthalmological examination and photographic recording of the eye conditions were carried out (days 1, 3, 7, then once every 3 days during the first month after surgery, and after the first month once a month). During the examination, intraocular pressure was measured to compare to normal range of 10 to 24 mm Hg (Tonovet, iCare, Finland). Signs of secondary cataracts were revealed using slit biomicroscopy (SL 17, Kowa Company, Ltd., Japan), the condition of the fundus was studied ophthalmoscopically (Omega 500, Heine Optotechnik GmbH & Co KG, Germany), additionally determining the capsule transparency. Depending on the severity of the changes, secondary cataracts of grade I, II or III were diagnosed. In grade I, the fundus was well ophthalmoscoped, in grade II, local opacities appeared in the field of view; in grade III, fundus details were poorly visible or the fundus was not visualized.

Rabbits were removed from the experiment by medical euthanasia, 2 and 4 animals 2 and 6 months after surgery, respectively. The eyeballs were enucleated and placed in a 10% neutralized formaldehyde solution. For a macroscopic assessment of the condition of the lens capsule, a section was made in the area of the dentate line (ora serrata) involving the retina. Most of the vitreous was aspirated. After photographing (a Huawei P30 smartphone, Huawei Technologies Co., Ltd., China), a sagittal section of the eye was made and its histological examination was carried out. After fixation in a 10% formalin solution, the samples were embedded in paraffin according to the common technique. Sections were prepared (a rotary automated microtome HM-325, MICROM International GmbH, Germany) and stained with hematoxylin and eosin to identify the general morphology and by Van Gieson to identify connective tissue and lens components. To study histological sections, a Jenamed-2 light microscope (Carl Zeiss Jena, Germany) combined with an ImageScope C digital microscopy system (Systems for Microscopy and Analysis LLC, Russia) was used.

All manipulations were carried out in accordance with Directive 2010/63/EU of the European Parliament and the Council of the European Union for the protection of animals used for scientific purposes (Strasbourg, September 22, 2010).

Results. In group 1, on day 2 after surgery, intraocular pressure reduced to 8.5±1 mm Hg. Its normalization occurred on day 3. After 2 months, opacification of the capsule corresponded to grade I (83.3% of cases) and grade II (16.7% of cases) cataracts. Macroscopically, after opening the eyeball, the lens was clearly identified, the lens capsule had a typical anatomical position (Fig. 1, A), and the opacification was located mainly in the equatorial zone. Microscopically, in the area of opacification, we found a significant growth of the lens substance between the layers of the capsule (see Fig. 1, B). Signs of chronic inflammation were visualized in the ciliary body and iris, and the pigment tissue of the ciliary body adhered to the capsule, forming adhesion that spread over its surface (see Fig. 1,

C). Six months after surgery, fundus ophthalmoscopy was difficult in 25% of cases. The lens capsule retained its typical location; the intraocular lens was clearly visible (see Fig. 1, D). In the long-term period, more pronounced thickening, compaction and opacification of the capsule occurred. Turbidity occupied a significant part of the surface, more pronounced in the equatorial zone. Foci of thickening and accumulations of lens cells between the layers of the capsule were microscopically detected (see Fig. 1, E, F).

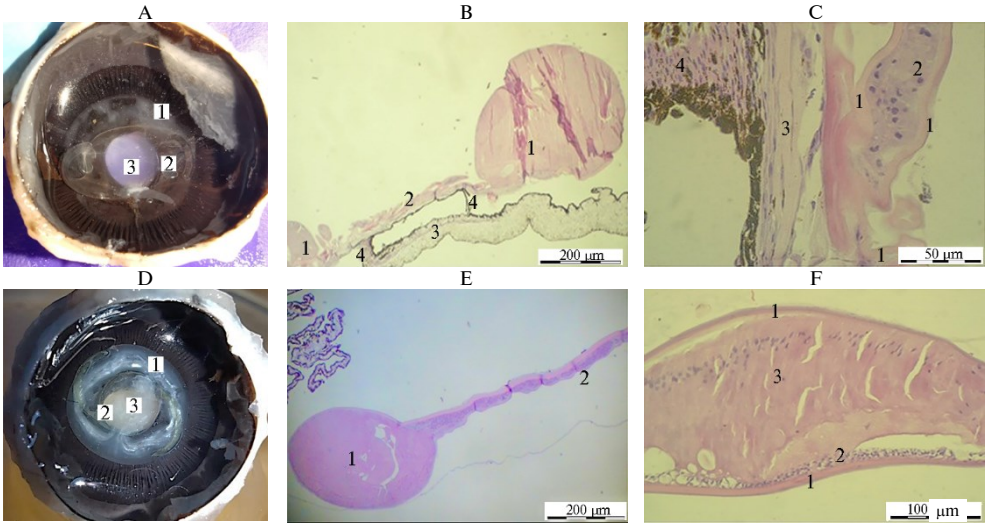

Fig. 1. The lens capsule of rabbits (*Oryctolagus cuniculus domesticus*) after phacoemulsification and implantation of an intraocular lens into the capsular bag: A — opened eyeball 2 months after surgery (1 — lens capsule with secondary cataract, 2 — intraocular lens, 3 — pupillary zone); B — microstructure of the lens capsule, general view (1 — area of thickening of the capsule, 2 — area of thinning with slight proliferation of lens epithelial cells between the layers of the capsule, 3 — iris, 4 — adhesions with the iris); C — fragment of the thickening focus (1 — capsule, 2 — epithelial cells of the lens, 3 — connective tissue capsule, 4 — pigment tissue of the iris); D — opened eyeball 6 months after surgery (1 — lens capsule with secondary cataract, 2 — intraocular lens, 3 — pupillary zone); E — microscopic changes in the lens capsule (1 — area of capsule thickening, 2 — area of thinning with accumulations of lens epithelial cells between the layers of the capsule); F — fragment of the thickening area (1 — capsule, 2 — proliferation of epithelial cells of the lens, 3 — lens fibers, signs of edema are observed) (hematoxylin and eosin staining, Jenamed-2 microscope, Carl Zeiss Jena, Germany; B, E: lens ×4, eyepiece ×10; B: objective ×40, eyepiece ×10; E: objective ×10, eyepiece ×10).

In group 2, during the postoperative period, intraocular pressure on day 2 reduced (8.6±1.2 mm Hg), in one case, ocular hypertension and iris bombardment were observed. Within 2 weeks, intraocular pressure returned to normal, but posterior synechiae of the iris remained. After 2 months, the secondary cataract (grade II) developed in 66.7% of cases, while it spread mainly in the equatorial zone (Fig. 2, A) and did not interfere with ophthalmoscopy. Microscopically, in the area of turbidity, numerous thickenings were revealed, formed by epithelial cells and lens fibers, in which signs of fragmentation, edema, and vacuolar degeneration of the lens epithelium were visible (see Fig. 2, B, C). In the ciliary body and iris, signs of chronic inflammation were identified as moderate marcrophage-lymphocytic infiltration. In the optically transparent area, only the lens capsule was observed in the form of acellular, weakly basophilic, fine-fibrous substance.

After 6 months, secondary cataracts were detected (see Fig. 2, D), complicating ophthalmoscopy in 50% of cases, in 25% the fundus was not visualized due to adhesions in the pupillary zone. Microscopic examination revealed an intracapsular ring; a small number of lens cells and lens fibers were observed between its material and the capsule (see Fig. 2, E). Nearby, encapsulated material of the

ring was revealed, and the capsule formed an adhesion with a thick layer of connective tissue (see Fig. 2, F). Signs of chronic inflammation were recorded in the iris and ciliary body: proliferation of connective tissue on the side of the iris, macrophage-lymphocytic infiltration, and edema.

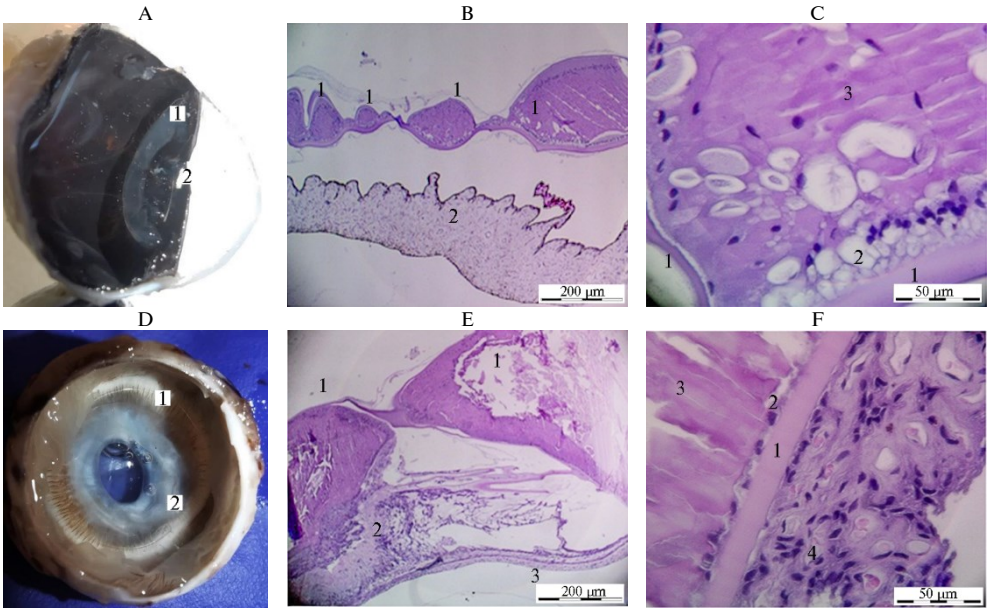

Fig. 2. The lens capsule of rabbits (*Oryctolagus cuniculus domesticus*) after phacoemulsification and implantation of an intracapsular ring: A — opened eyeball 2 months after surgery (1 — secondary cataract, 2 — pupillary zone); B — microstructure of the lens capsule, general view (1 — numerous thickenings of different sizes, 2 — iris); C — fragment of the thickening area (1 — capsule, 2 — epithelial cells of the lens, many in a state of hydrooptic dystrophy, 3 — lens fibers); D — opened eyeball 6 months after surgery (1 — intracapsular ring, displaced outside the capsular bag, 2 — equator of the capsular bag with secondary cataract); E — microscopic changes in the lens capsule (1 — areas of thickening in the lens capsule, 2 — connective tissue capsule formed around the ring, 3 — fibrous membrane, 4 — intracapsular ring); F — marginal zone of the thickening focus (1 — capsule, lens epithelium, 2 — lens fibers, 3 — connective tissue growing to the capsule from the side of the iris) (hematoxylin and eosin staining, Jenamed-2 microscope, Carl Zeiss Jena, Germany); C, F: objective ×4, eyepiece ×10; B, E: objective ×40, eyepiece ×10).

In group 3, intraocular pressure on day 2 was low (9.0±1.5 mm Hg) and normalized by day 5. After 2 months, the fundus of the eye was well visualized by ophthalmoscopy in all animals. At autopsy, secondary cataract grade I was revealed in the equatorial zone; an optically transparent "window" was preserved in the central zone in the area of the posterior capsulorhexis (Fig. 3, A). In the area of opacity, foci of thickening were identified, formed by lens epithelial cells and fibers inside the lens capsule (see Fig. 3, B, C). An optically transparent space was visualized in the pupil projection. The ciliary body and iris showed signs of chronic inflammation.

Six months after the surgery, all animals showed signs of secondary cataract, more pronounced in the equatorial zone, but it did not interfere with ophthalmoscopy, since the central zone where capsulorhexis was performed remained optically transparent (see Fig. 3, D). Microscopically, in the area of opacification, abundant deposition of lens fibers between the layers of the capsule was revealed (see Fig. 3, E). Signs of destruction, fragmentation, and edema were identified in the lens fibers, and the adjacent lens capsule locally formed folds (see Fig. 3, F). Signs of chronic inflammation were identified in the ciliary body and iris. The capsule structures were not identified in the pupil projection.

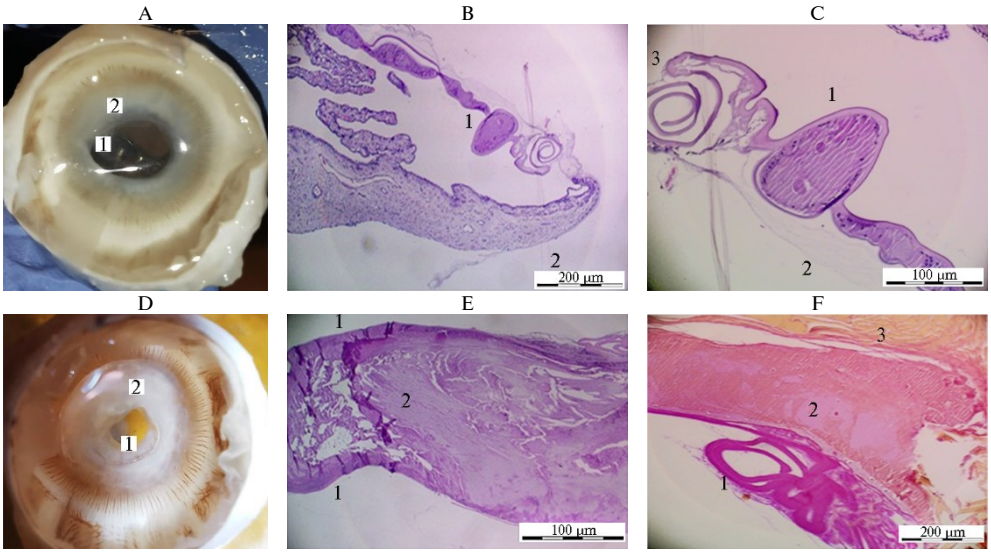

Fig. 3. The lens capsule of rabbits (*Oryctolagus cuniculus domesticus*) after phacoemulsification and capsulorhexis of the posterior capsule of the lens: A — opened eyeball 2 months after surgery (1 — optically transparent central part of the capsule, 2 — area of secondary cataract); B — microstructure of the capsule, general view (1 — numerous foci of thickening, 2 — iris); C — fragment of the cataract area (1 — thickening area, lens epithelium and fibers, inside the lens capsule, 2 — connective tissue capsule, 3 — capsule folds); D — opened eyeball 6 months after surgery (1 — optically transparent central part of the capsule, 2 — area of secondary cataract); E — area of turbidity (1 — capsule leaves, 2 — lens fibers); F — microscopic changes in the lens capsule (1 — folds of the capsule, 2 — lens fibers) (staining with hematoxylin and eosin, Jenamed-2 microscope, Carl Zeiss Jena, Germany; B, E: objective ×4, eyepiece ×10; C, F: objective ×10, eyepiece ×10).

Based on the studies, it was established that within 2 days after the sugery, all animals experienced intraocular inflammation, one of the characteristics of which was a decrease in intraocular pressure. During the postoperative period, secondary cataracts developed in all animals, which corresponds to the data of I.D. Bras et al. [4], however, its severity turned out to be different in the studied groups, for example, after capsulorhexis of the posterior surface of the lens capsule, it was minimal. Without intraoperative preventive measures, secondary cataracts of grades II-III develop in 16.7% rabbits within 2 months and in 100% rabbits within 6 months [1]. The technique of intraoperative prophylaxis changed the situation. According to our results, 2 months after phacoemulsification combined with implantation of an intracapsular ring, this complication occurred in 66.7% of cases, after implantation of an intraocular lens in 16.7%, and did not develop after capsulorhexis of the posterior capsule of the lens. After 6 months, the process progressed and was recorded in 75% rabbits after implantation of an intracapsular ring, in 25% after implantation of an intraocular lens, while did not occur after posterior capsulorhexis.

In morphological studies, we obtained results consistent with the data of A. Morales et al. [15] and indicating that secondary cataract is associated with the proliferation of epithelial cells and the formation of lens fibers. Moreover, our data show that the formation of fibrous tissue on the surface of the capsule is not associated with metaplasia of the lens epithelium, but with the involvement of the iris in inflammation. The iris adheres to the lens capsule and forms adhesions with it. This information complements modern ideas about the pathogenesis of secondary cataracts.

Our studies have shown that the most effective intraoperative method for the prevention of secondary cataracts, which is advisable to use in combination with phacoemulsification, is capsulorhexis of the posterior lens capsule (Table).

This is evidenced by the minimal, compared to other methods, severity of postoperative structural changes (e.g., proliferation of epithelial cells, formation of lens fibers, development of adhesions) and maintaining the optical transparency of the central zone of the capsule located in the projection of the pupil. Thereof, posterior capsulorhexis can restore visual functions due to a better clinical effect than with implantation of an intraocular lens and intracapsular ring.

Proportion (%) of rabbits (*Oryctolagus cuniculus domesticus*) with secondary cataracts upon intraoperative prevention ($n = 18$)

Group	2 months after surgery			6 months after surgery		
	grade I	grade II	grade III	grade I	grade II	grade III
1	83.3	16.7	–	75.0	25.0	–
2	33.3	66.7	–	25.0	50.0	25.0
3	100	–	–	100	–	–

N o t e. For a description of groups and sample sizes by group and observation period, see the Materials and methods section. Dashes mean that secondary cataract of the grade did not occur.

Thus, secondary cataract is a common complication after phacoemulsification in rabbits. Its pathogenesis is based on the proliferation of lens epithelial cells with the formation of structurally and functionally defective lens fibers. In addition, an adhesive process occurs, the iris or ciliary body, being involved in inflammation, form adhesions with the capsular bag of the lens. The results of studying the pathogenesis of secondary cataracts allow us to conclude that capsulorhexis of the posterior lens capsule is an effective intraoperative technique for preventing secondary cataracts that can be used in combination with phacoemulsification. This technique causes minimal structural changes in the capsule bag, while the central zone of the capsule located in the projection of the pupil remains optically transparent. This helps restore visual function and allows for a positive clinical effect.

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