DOI: 10.1590/1809-6891v24e-76196E

Ciência Animal Brasileira
Brazilian Animal Science

e-ISSN 1809-6891

Section: Veterinary medicine
Research article

Ex vivo evaluation of anterior lens capsule staining in horses with three concentrations of gentian violet for surgical training

Avaliação *ex vivo* da coloração da cápsula anterior da lente de cavalos com três concentrações de violeta genciana para treinamento cirúrgico

Marcelle Bettio[1], Maiara Poersch Seibel[1*], Maria Eduarda Mattos Franceschini[1], Rafaella Silva Rocha[1], Renata Lima Baptista[1], Anita Marchionatti Pigatto[2], João Antonio Tadeu Pigatto[1]

[1]Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil
[2]Universidade Estadual Paulista "Júlio de Mesquita Filho (UNESP), Botucatu, São Paulo, Brazil
[*]Corresponding author: bettio.marcelle@gmail.com

Abstract
The aim of this study was to evaluate and compare the effectiveness of three concentrations of gentian violet (0.5%, 0.1% and 0.05%) for staining the anterior capsule of the lens in horses. Thirty-six *post-mortem* equine eyes were collected. The eyes were subdivided into three groups composed of 12 eyes each, according to the concentration of gentian violet used. The effectiveness of staining the anterior capsule of the lens with different concentrations of gentian violet was assessed using an empirical system of evaluation on adequate or inadequate staining of capsular flaps. Based on the evaluation of the examiner, the 0.1% and 0.05% concentrations of gentian violet allowed adequate visualisation of the anterior capsule for continuous curvilinear capsulotomy training, whereas the 0.5% concentration produced strong and inadequate capsular staining. The model developed using gentian violet at concentrations of 0.1% and 0.05% allowed a clear visualisation of the capsular flap, which makes it viable as a model for training the continuous curvilinear capsulotomy step in cataract surgery in horses.
Keywords: anterior capsulotomy; vital dyes; equine; wet lab; surgical training

Resumo
O objetivo deste estudo foi avaliar e comparar a eficácia de três concentrações de violeta genciana (0,5%, 0,1% e 0,05%) na coloração da cápsula anterior da lente em equinos. Trinta e seis olhos de equinos *post-morten* foram utilizados. De acordo com a concentração de violeta genciana utilizada, os olhos foram subdivididos em três grupos compostos por 12 olhos cada. A avaliação da eficácia em coloração da cápsula anterior da lente com diferentes concentrações de violeta de genciana foi realizada por meio de um sistema empírico de avaliação da coloração adequada ou inadequada de retalhos capsulares. Com base na avaliação dos examinadores, as concentrações de 0,1% e 0,05% de violeta de genciana permitiram a visualização adequada da cápsula anterior para o treinamento da capsulotomia curvilínea contínua enquanto a concentração de 0,5% produziu uma coloração capsular forte e inadequada. O modelo desenvolvido com violeta genciana, nas concentrações de 0,1% e 0,05%, permitiu a visualização nítida do retalho capsular, o que o torna viável como modelo para treinamento da etapa de capsulotomia curvilínea contínua em cirurgia de catarata em equinos.
Palavras-chave: capsulotomia anterior; corantes vitais; equino; laboratório úmido; treinamento cirúrgico

1. Introduction

Phacoemulsification has become the recommended surgical procedure for treating cataracts[1,2,3]. Performing an adequate anterior capsulotomy is a crucial step in this technique[2,4,5,6,7,8]. However, the proper visibility of the anterior lens capsule to create an ideal capsulorhexis may be compromised in eyes with mature cataracts[4,8,9,10,11,12,13].

In ophthalmic surgeries, the use of vital dyes has become an effective and useful tool for the visualisation of target tissues[2]. The application of these products for the selective staining of the anterior capsule of the lens enables the proper identification of this structure[8,11,14], allowing greater control in capsulorhexis. Furthermore, easy capsular identification is important for surgeons who are on the learning curve of this technique[4,15].

Continuous curvilinear capsulotomy (CCC) is among the most technically challenging manoeuvres to master during phacoemulsification[16]. A poorly performed capsulotomy can lead to serious intra and postoperative complications[2,4,5,6,7,8]. In human ophthalmology, several studies have been carried out with the aim of evaluating different vital dyes for staining the anterior capsule of the lens, exploring a wide range of concentrations and analysing their

Received: May 21, 2023. Accepted: July 31, 2023. Published: August 28, de 2023.

harmful effects on intraocular structures[8,15,20,21].

To the authors' knowledge, there are no studies evaluating gentian violet for staining the anterior capsule of the lens in horses. Thus, aiming to facilitate the identification of the anterior capsule of the lens for CCC training, the efficacy of three concentrations of gentian violet (0.5%, 0.1% and 0.05%) in horse eyes was investigated *ex vivo*.

2.Materials and methods

Thirty-six eyes of 18 male or female horses (*Equus caballus*) of different ages and breeds, obtained from a slaughterhouse (Foresta, São Gabriel, RS, Brazil), were used. The animals were slaughtered for reasons unrelated to this study. The research was approved by the Research Committee of the College of Veterinary of the Federal University of Rio Grande do Sul. The eyes were enucleated immediately after slaughter and kept in a humid chamber for 2 h until the experiment was carried out. All eyes underwent ophthalmic examination, which included slit-lamp biomicroscopy (Portable slit lamp, Kowa SL 15, Nagoya, Japan) and a fluorescein test (1% sodium fluorescein, Allergan®). All equine eyes used in this study had a transparent crystalline lens and showed no noteworthy ocular alterations.

Three concentrations (0.5%, 0.1% and 0.05%) of gentian violet (gentian violet, ethyl alcohol, purified water; Gentian Violet 2% solution, Needs® 30 mL) were used for staining the anterior capsule of the lens. The concentrations were prepared as follows: 0.5%: dilution of 1 mL of dye in 3 mL of Ringer lactate; 0.1%: 1 mL dye in 19 mL lactated Ringer's solution; 0.05%: 1 mL dye in 39 mL lactated Ringer's solution.

The eyes were randomly assigned to three groups, consisting of 12 eyes on each, based on the dye concentration: Group A (0.5%), Group B (0.1%) and Group C (0.05%). Each eye was fixed on Styrofoam with the aid of pins, under a surgical microscope (DFVasconcellos MU-M19, Rio de Janeiro, Brazil). All staining and CCC executions were performed by the same surgeon. Under the operating microscope, an incision in the clear cornea, with a 2.75-mm corneal scalpel, was performed to access the anterior chamber. Through the incision, a large air bubble was injected, using a 27-gauge (G) cannula connected to a 3-mL syringe, to reconstruct the anterior chamber. With the 27-G cannula connected to a syringe containing dye, 0.4 mL of the respective gentian violet concentration was gently injected below the air bubble on the surface of the capsule. One minute was allowed for dye impregnation; subsequently, the anterior chamber was irrigated with lactated Ringer's solution to remove the remaining dye. Afterwards, the cornea was dissected through a 360-degree limbic incision 2 mm from the sclera, and the iris was removed to facilitate the visualisation of the capsule and the execution of the CCC (Figure 1).

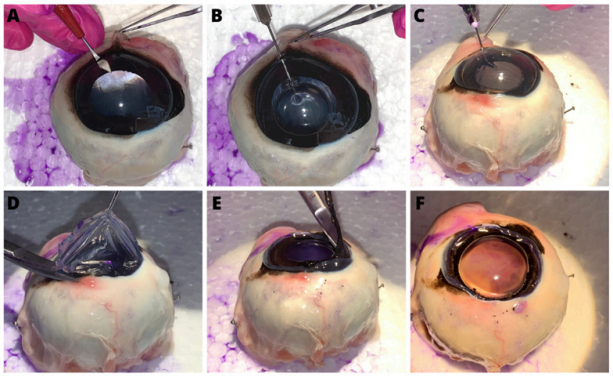

Figure 1. Staining of the anterior capsule of the equine lens with gentian violet solution at 0.05% concentration. (A) incision in the clear cornea with a 2.75-mm scalpel; (B) injection of a large air bubble into the anterior chamber; (C) 0.4 mL of dye injected into the anterior chamber between the anterior capsule and the air bubble; (D) excision of the corneoscleral button; (E) removal of the iris; (F) impregnation of the anterior capsule with 0.05% gentian violet dye.

Continuous curvilinear capsulotomy was performed after excision of the corneoscleral button (D) and the removal of the iris (E) in the open configuration (without the anterior chamber), using the magnification of the surgical microscope and a cystotome. This instrument was equipped with a 26-G bent needle. The manoeuvre started with a perforation in the centre of the anterior capsule with the cystotome to raise a capsular flap. Afterwards, the edge of this flap was held, tearing the capsule clockwise or counter-clockwise until reaching the opposite side of the initial incision (Figure 2).

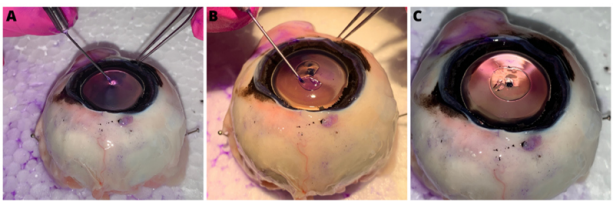

Figure 2. Continuous curvilinear capsulotomy technique after staining the anterior capsule with a 0.05% gentian violet solution and removing the corneoscleral button and the iris. (A) perforation in the centre of the anterior capsule with the help of a cystotome; (B) raised capsular flap, tearing it counter-clockwise until reaching the initial incision on the opposite side; (C) completion of the continuous curvilinear capsulotomy.

The CCC was performed with the three prepared gentian violet concentrations (Figure 3).

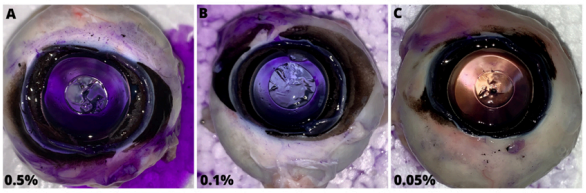

Figure 3. Continuous curvilinear capsulotomy with three concentrations of gentian violet. (A) 0.5%, (B) 0.1%, (C) 0.05%.

The samples consisted of capsular flaps obtained right after the staining technique and CCC preparation. These anterior capsulotomies were placed under a glass slide and positioned on a lined paper, with the respective solution beside it, and then photographed with a digital camera (Canon EOS REBEL T3i digital SLR, exposure time 1/60s and ISO 400) (Figure 4). The lined paper served as an adjuvant for the examiners' analysis. The white background of the paper created contrast for the dye, and the lines contributed to differentiating the degree of intensity of the samples through their visibility.

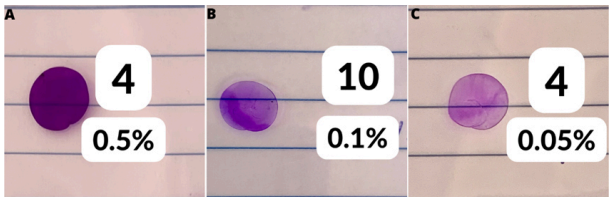

Figure 4. Macroscopic evaluation of the capsular flap with different concentrations of gentian violet. (A) 0.5%, (B) 0.1%. (C) 0.05%.

The effectiveness of staining the anterior lens capsule with different concentrations of gentian violet (0.05%, 0.1% and 0.5%) was graded according to an empirical evaluation system, by two examiners experienced in surgery of phacoemulsification, in adequate or inadequate staining. This analysis took place after all samples had been photographed. The evaluators analysed the images randomly and blindly. The images were manually shuffled. After being identified with numerals from 1 to 36, the subtitles of the samples and their respective concentrations were added to a Microsoft-Excel 2013 software table. Afterwards, the concentration of the samples of all the photographs was hidden. Thus, the examiners did not know to which group the sample belonged. The analyses were performed using IBM-SPSS for Windows version 22.0 software and tabulated using Microsoft-Excel 2013 software, and the tests were performed with a significance level of 5%.

3. Results

All concentrations of gentian violet (0.5%, 0.1% and 0.05%) were able to stain the anterior lens capsule, making it possible to perform an anterior capsulotomy in all 36 eyes. However, only 25 capsular flaps were adequately classified by the examiners. From the cross-data between the evaluators, the percentage of agreement was 98.2%, with only one divergent sample, with the reproducibility/agreement between them being almost perfect (Kappa = 0.936) (Table 1).

Table 1. Description of the results of the classification of staining, inadequate or adequate, among the examiners, and results of the coefficient of reproducibility/agreement between them.

	Examiner 1	Examiner 2	Kappa (CI: 95%)
Inadequate	11 (30.6)	12 (33.3)	0.936
Adequate	25 (69.4)	24 (66.67)	(0.813; 1.000)
Total	36 (100)	36 (100)	

Values are expressed as n (%)

Table 2 demonstrates the statistically significant association between the dye concentrations and the adequacy of staining the capsular flaps for both examiners ($p < 0.001$), with the 0.5% concentration of gentian violet being the only one that yielded inadequate results for both examiners.

Table 2. Description of the adequacy of the results according to the dye concentration for each examiner and the results of the association tests.

Result	Concentration			P
	0.05	0.1	0.5	
Examiner 1				**<0.001**
Inadequate	0 (0)	0 (0)	11 (91.7)	
Adequate	12 (100)	12 (100)	1 (8.3)	
Examiner 2				**<0.001**
Inadequate	0 (0)	0 (0)	12 (100)	
Adequate	12 (100)	12 (100)	0 (0)	
Total	**12 (100)**	**12 (100)**	**12 (100)**	

Values are expressed as n (%); Likelihood ratio test

All samples from anterior capsulotomies stained with 0.1% and 0.05% gentian violet were classified as adequately stained for surgical practice by both examiners. The 0.5% concentration had only one sample categorised by Examiner 1 as adequate, not representing a significant percentage.

4. Discussion

Vital stains have become essential tools to improve the visualisation of the anterior lens capsule, and their use is now well-established and widespread[2]. Different types of dyes, including trypan blue, indocyanine green,

fluorescein sodium, gentian violet[8,15,20], methylene blue[20] and brilliant blue[21], have been proposed and tested. However, for safety reasons, only trypan blue is approved for *in vivo* use[2,20]. In the present study, the choice of gentian violet vital dye was justified by the *ex vivo* use in animals, and therefore, its toxicity was not taken into account.

Gentian violet is a dye with an extensive and diverse history as a medicinal agent[24,25], mainly due to its antiseptic action[26]. In ophthalmology, there are reports of its use as a marker for the cornea, conjunctiva and anterior lens capsule[11]. We chose to use this dye for this study because it is an easily obtainable solution that is commercially available in drugstores, with a low cost, simple to dilute and an intense colour.

The use of dyes in the anterior capsule of the lens is also beneficial, and the CCC technique is employed in wet laboratories (wet lab). Wet labs are surgical training models that use animal eyes, *in vivo* or *ex vivo,* or human cadavers. Although there are different models of CCC surgical practice, Pujari et al.[19] defend the wet lab training model as it helps the learning curve and increases the operative skills and safety of surgeons. In agreement, Moharana et al.[27] classify the use of wet labs associated with vital staining as a highly reliable method to mimic the surgical procedure. In addition, this approach is a cheap, applicable and reproducible model of surgical practice. Thus, we evaluated three concentrations of gentian violet (0.5%, 0.01% and 0.05%) on the anterior capsule of the *post-mortem* equine lens to demonstrate adequate concentrations of this dye for carrying out the surgical practice of CCC in an experimental laboratory.

Different techniques for staining the lens capsule are described. Fernández et al.[28] injected the dye under an air bubble, as originally described by Melles et al.[10]. In the present study, we opted for the air bubble staining technique as this method has previously been described in phacoemulsification training studies[10,28]. In addition, it is an easy, economical, reproducible technique that facilitates the homogeneous staining of the capsule. The adequate exposure time of the anterior capsule to the vital dye has not yet been established. In previous studies, exposure periods of 10 s[14], 30 s[28] and 60 s[11,20] were established. In our study, the contact time between the dye and the anterior capsule was 60 s. The divergence between the exposure duration published in previous studies may be due to the staining method used or the vital dye chosen.

In the study carried out by Dong et al.[18] the groups and subgroups were divided according to the experience in manipulating the capsulorhexis and the increasing difficulty in training the device, respectively. In subgroup 01, the device had no cover, which represented the cornea in the system. Dong et al.[18] state that this condition is suitable for surgeons without any experience as it facilitates the perception of performing a continuous curvilinear capsulotomy, as well as the acquisition of fine motor coordination. In our experiment, as training was performed in *post-mortem* eyes, we chose to prepare the CCC without the presence of the cornea and iris. This choice was made due to the lack of mydriasis in the eyes and to facilitate the training of the inexperienced surgeon. This methodology has already been used in another study and proved to be effective[11].

The capsulotomy was performed with a cystotome made from a 26-G bent needle. The use of this instrument is not universal; however, it is a simple, cheap and easy-to-prepare alternative and has successfully been used in other experiments and in surgical routine[29]. In addition, Plummer[30] comments that because horses have a larger eyeball, a shallow anterior chamber and a small corneal incision for access, the standard Utrata forceps or the elongated ones do not allow the execution of the technique. This makes the use of suitable capsulorhexis forceps for horses necessary, which makes training more expensive.

Trypan blue is the only dye approved for *in vivo* use because it is effective and safe and reliably and selectively stains the anterior capsule[2,20]. When its use is directed towards surgical training, cost-effectiveness is not advantageous: it is a material for hospital use, which makes acquisition difficult, in addition to existing solely in bottles of only 1 mL and being sold only in boxes of 10 bottles, rendering training more expensive.

The use of gentian violet to stain the capsule was proposed in 1998. Since then, studies have been carried out to evaluate the efficacy of the dye and its safety for intraocular use. However, in the present study, we did not take into account the dye toxicity as the purpose of the experiment was to identify adequate concentrations of the dye under the anterior capsule of the lens to assist in surgical training for CCC in wet labs. We therefore opted for the 2% gentian violet solution, sold in drugstores for topical antiseptic use, due to the easy availability of this product.

Eldin et al.[9] evaluated concentrations of 0.05% to 2% in an *ex vivo* study in rabbits, obtaining satisfactory results with all concentrations. Other investigators used 0.01% and 0.001% concentrations to stain rat and human anterior capsules and concluded that the 0.01% concentration provided a better visualisation of the capsule. Chang et al.[20] examined the staining potential of different types of dyes, including gentian violet at concentrations of 0.001%, 0.01%, 0.1% and 1% for 1 min, to stain the rabbit anterior capsule and concluded that the minimum concentration required to produce effective staining was 0.01%. Another *in vitro* study compared 13 vital dyes for capsule staining, including gentian violet at concentrations of 0.5% and 0.05%. The authors reported that the 0.5% concentration produced

moderate staining, and the 0.05% concentration produced light staining[11].

In the present study, in contrast to these results, adequate staining for surgical practice was found with concentrations of 0.1% and 0.05%, and staining classified as inappropriate was observed for the 0.5% concentration because it caused excessive staining, which may make it difficult to clearly differentiate the capsule and cortex during capsulorhexis. An inadequate classification of high concentrations of gentian violet has already been described in another study[20], which corroborates the results of the present study.

Some explanations for the divergent results observed in the literature include the time of exposure of the capsule to the dye in each experiment; the technique used to apply the dye; different existing purities of the gentian violet products used, since some studies use powder dye; and anatomical differences of the anterior capsules of different species used as models in the studies. Therefore, clinical studies that standardise staining methods and exposure times are needed.

Methods for analysing and classifying the intensity of staining of the anterior lens capsule are not pre-established. Macroscopic and subjective evaluations to assess whether the staining is adequate have been made in previous studies[11,14]. In the study by Wilinska et al.[14], in addition to evaluating the toxicity of the solutions, the analysis of the staining of the samples by photodocumentation, evaluating the contrast between the stained capsule and the cortex, was one of the criteria taken into account to determine an effective staining. Chang et al.[20], in turn, used a semi-quantitative scoring system to assess staining effectiveness, where zero represented low contrast between capsule and cortex and four represented excellent contrast. Evaluators were represented by surgeons experienced in cataract surgery. Fernández- Bueneo[28] evaluated the colour macroscopically and microscopically and stated that macroscopic evaluation is a quick and useful method for surgeons to choose a product to use during daily surgeries. In the present study, we opted for the macroscopic and subjective evaluation of two examiners with surgical experience. The analysis was performed blindly and at random, and after that, the data were crossed. The choice of the number of examiners was made to increase the fidelity of the study. The choice of method was based on the intention of an analysis that reproduced the real surgical environment.

5. Conclusions

Based on the results, the gentian violet concentrations of 0.1% and 0.05% were adequate for staining the anterior capsule of the lens in horses. The present study contributes to training in cataract surgery in equines.

Conflict of interests

The authors declare no conflict of interest.

Author contributions

Conceptualization: M. Bettio and J.A.T. Pigatto. *Methodology*: M. Bettio, M.P. Seibel, M.E.M. Franceschini, R.S. Rocha, R. Baptista, A.M. Pigatto and J.A.T. Pigatto. *Investigation*: M. Bettio and J.A.T. Pigatto. *Project administration*: J.A.T. Pigatto. *Writing (original draft, review & editing):* M. Bettio and J.A.T. Pigatto.

Acknowledgements

The authors would like to thank Avisui food Ltd for supplying the eyes used in this research free of charge, and “Coordination for the Improvement of Higher Education Personnel” (CAPES), which provided the fellowship grant for one of the authors (M.B.).

References

1 McMullen Junior RJ, Utter ME. Current developments in equine cataract surgery. Equine Veterinary Journal. Supplement. 2010;37:38-45. Available from: doi: http://doi.org/10.1111/j.2042-3306.2010.tb05633.x.

2 Jhanju V, Chan E, Das S, Zhang H, Vajpayee RB. Trypan blue dye for anterior segment surgeries . Eye (Lond) 2011;25(9):1113-1120. Available from: doi: http://doi.org/10.1038/eye.2011.139.

3 McMullen RJ, Stoppini R. Diseases and surgery of the lens. In: Gilger BC, editor. Equine Ophthalmology. 4th ed. Hoboken : John Wiley & Sons; 2017. p.416-452. Available from: doi: http://doi.org/10.1002/9781119782285.ch7.

4 Jacobs DS, Cox TA, Wagoner MD, Ariyasu RG, Karp CL. Capsule staining as an adjunct to cataract surgery. Ophthalmology. 2006;113(4):707-713. Available from: doi: http://doi.org/10.1016/j.ophtha.2006.01.024.

5 Hassaballa MAM, Osman AAEL. Delineating the extent of anterior capsulorhexis with gentian violet using capsulorhexis marker: a preliminary study of efficacy and toxicity in an animal model. Clinical Ophthalmology. 2011;5:831-836. Available from: doi: http://doi.org/10.2147/OPTH.S21791.

6 Haeussler-Sinangin Y, Dahlhoff D, Schultz T, Dick HB. Clinical performance in continuous curvilinear capsulorhexis creation supported by a digital image guidance system. Journal of Cataract and Refractive Surgery. 2017;43(3):348-352. Available from: doi: http://doi.org/10.1016/j.jcrs.2016.12.027.

7 Hu WF, Chen SH. Advances in capsulorhexis. Current Opinion Ophthalmology. 2019;30(1):19-24. Available from: doi: http://doi.org/10.1097/ICU.0000000000000539.

8 Sharma B, Abell RG, Arora T, Antony T, Vajpayee RB. Techniques of anterior capsulotomy in cataract surgery. Indian Journal of Ophthalmology. 2019;67(4):450-460. Available from: doi: http://doi.org/10.4103/ijo.IJO_1728_18.

9 Eldin SAG, Mehelmy EM, Shazli EM, Mostafa YM. Experimental staining of the anterior lens capsule in albino rabbits. Journal of Cataract and Refractive Surgery. 1999;25(9):1289-1294. Available from: doi: http://doi.org/10.1016/s0886-3350(99)00153-4.

10 Melles GRJ, Waard PW, Pameyer JH, Beekhuis WH. Trypan blue capsule staining I'm visualizethe capsulorhexis in cataract surgery. Journal of Cataract Refractive Surgery. 1999;25(1):7-9. Available from: doi: http://doi.org/10.1016/s0886-3350(99)80004-2.

11 Rodrigues EB, Costa EF, Penha FM, Melo GB, Bottós J, Dib E, et al. The use of vital dyes in ocular surgery. Survey of Ophthalmology. 2009;54(5):576-617. Available from: doi: http://doi.org/10.1016/j.survophthal.2009.04.011.

12 Andjelić S, Zupančič G, Hawlina M. The effect of gentian violet on human previous lens epithelial cells. Current Eye Research. 2014;39(10):1020-1025. Available from: doi: http://doi.org/10.3109/02713683.2014.894077.

13 Simsek C, Gokmen O. The effects of vital dyes on mechanic properties of the human previous lens capsule. Indian Journal of Ophthalmology. 2020;68:66-70. Available from: doi: http://doi.org/10.4103/ijo.IJO_285_19.

14 Wilińska, J, Mocanu, B, Awad, D, Gousia, D, Hillner, C, Brannath, W, et al. New stains for anterior capsule surgery. Journal of Cataract and Refractive Surgery. 2019;45(2): 213–218. Available from: http://doi.org/10.1016/j.jcrs.2018.09.016.

15 Dada VK, Sharma MDM, Sudan R, Sethi H, Dada T, Pangtey MS, et al. Anterior capsule staining for capsulorhexis in cases of white cataract. Journal of Cataract and Refractive Surgery. 2004;30(2):326-333. Available from: doi: http://doi.org/10.1016/S0886-3350(03)00573-X.

16 Prakash G, Jhanji V, Sharma N, Gupta K, Titiyal JS, Vajpayee RB. Assessment of perceived difficulties by residents in performing routine steps in phacoemulsification surgery and in managing complications. Canadian Journal of Ophthalmology. 2009;44(3):284-287. Available from: doi: http://doi.org/10.3129/i09-051.

17 Oflaz AB, Köktekir, BE, Okudan S. Does cataract surgery simulation correlate with real- life experience? Turkish Journal of Ophthalmology. 2018;48(3):122-6. Available from: doi: http://doi.org/10.4274/tjo.10586.

18 Dong J, Yang X, Yang X, Li J. A practical continuous curvilinear capsulorhexis self-training system. Indian Journal of Ophthalmology. 2021;69(10):2678-286. Available from: doi: http://doi.org/10.4103/ijo.IJO_210_21.

19 Pujari A, Saluja G, Bhaskaran K, Modaboyina S, Asif MI, Agarwal T, et al. Animal and corpse human eyes for residents' surgical training in ophthalmology. Survey Ophthalmology. 2021;67(1):226-251. Available from: doi: http://doi.org/10.1016/j.survophthal.2021.05.004.

20 Chang YS, Tseng S, Tseng S. Comparison of dyes for cataract surgery: Part 2: Effectiveness of capsule staining in a rabbit model. Journal of Cataract and Refractive Surgery. 2005;31(4):799-804. Available from: doi: http://doi.org/10.1016/j.jcrs.2004.09.029.

21 Hisatomi T, Enaida H, Matsumoto H, Kagimoto T, Ueno A, Hata Y, et al. staining ability and biocompatibility of brilliant blue G. Archives of Ophthalmology. 2006;124(4):514-9. Available from: doi: http://doi.org/10.1001/archopht.124.4.514.

22 Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159-74 . Available from: https://doi.org/10.2307/2529310

23 Kirkwood BR, Sterne JAC. Essential medical statistics. 2nd ed. Massachusetts: Blackwell Science; 2006. Available from: https://bcs.wiley.com/he-bcs/Books?action=index&bcsId=11848&itemId=0865428719

24 Pona A, Quan, EY, Cline A, Feldman SR. Review of the use of gentian violet in dermatology practice. Dermatology Online Journal. 2020;26(5):13030. Available from: https://doi.org/10.5070/D3265048772

25 Prabha N, Arora RD, Ganguly S, Chhabra N. Gentian violet: revisited. Indian Journal of Dermatology Venereology and Leprology. 2020;86:600-603. Available from: doi: http://doi.org/10.4103/ijdvl.IJDVL_579_19.

26 Ünlü K, Askünger A, Söker S, Kilinç N, Karaca C, Erdinc M. Gentian violet solution for staining the previous capsule. Indian Journal of Dermatology Venereology and Leprology. 2020;86(5):600-603. Available from: doi: http://doi.org/10.1016/s0886-3350(00)00360-6.

27 Moharana B, Singh P, Patel S, Srivastava P, Sharma B. Commentary: High fidelity and cost-effective cataract surgery training system: need of the hour. Indian Journal of Ophthalmology. 2021;69(10):2686-2687. doi: http://doi.org/10.4103/ijo.IJO_1877_21.

(28) Fernandez-Bueno I, Usategui-Martín R, Pastor JC, Andrés-Iglesias C. Ex-vivo method to quantifiably the staining effectiveness of anterior lens capsule dyes. Translational Vision Science & Technology. 2021;10(17):1-6. Available from: http://doi.org/10.1167/tvst.10.14.17.

(29) Naik MP, Sethi H, Kasiviswanathan P. Modified bandage-contact-lens used as a guide-marker for performing continuous curvilinear capsulorhexis by a first year post graduate ophthalmology resident. American Journal of Ophthalmology Case Reports. 2020; 20:100889. Available from: doi: http://doi.org/10.1016/j.ajoc.2020.100889.

(30) Plummer EC. Equine ophthalmology. In: Gellat K. (org.). Veterinary Ophthalmology. 6th ed. New York: Wiley-Blackwell; 2021. Available from: https://www.wiley.com/en-us/Veterinary+Ophthalmology,+2+Volume+Set,+6th+Edition-p-9781119441830.