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Preservation Techniques of the Human Cadaveric Eye

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Abstract: Various preservation methods have been devised to prolong the storage of human cadavers that are donated for research and training purposes. However, the majority of these embalming solutions fail to maintain the structure of the human cadaveric bulbi oculi intact, as they have a tendency to become deflated and dehydrated post-mortem. In fact, as a result, many ophthalmic surgery tuition centers are currently resorting to the use of fresh animal eyes to aid their students in mastering ocular surgical techniques.
The objective of this literature review is to identify methods that warrant further investigation as they may aid in devising effective preservation techniques for the human cadaveric eye. Methods that can possibly be applied to increase the intra-ocular pressure and prevent deflation of the human cadaveric eye include: the administration of increased fluid injections to increase volume, application of the head-down tilt, cauthery or clamping of the optic nerve and ophthalmic vessels, induction of corneal rigidity as well as alteration of angle closure and cerebrospinal fluid pressure. Moreover, several corneal artificial hydration solutions exist that warrant further experimentation to establish if they can also be used to prevent dehydration of the human cadaveric eye. These include: hyaluronic acid or carboxymethylcellulose-based solutions, trehalose-based solutions, hydroxypropyl-guar or hypromellose-based solutions as well as hypotonic or isotonic saline.

Keywords: Preservation, cadaveric eye, human, intra-ocular pressure, intra-ocular volume.

Introduction:

Significant improvements in success rates of ophthalmic surgery are owed to advances in surgical techniques and instrumentation. There is no need to highlight the imperative role of intensive surgical training and continuous hands-on practice in reaching such impeccable standards. The formulation of effective preservation techniques for the human cadaveric eye for use in ocular surgery training is the next target milestone, considering that common acceptable preservation methods which do not markedly alter tissue structure such as Thiel embalming,[1] cryopreservation[2] or hypothermic storage,[3] as well as immersion in salt[4] or glycerol-based media[5] fail to maintain the structure of the human cadaveric eye intact. This deficiency currently leaves ophthalmic surgery tuition centres with no other option other than to facilitate their students' practice on bovine or porcine eyes.

Materials & Methods:

The literature search and selection criteria:

A literature search was conducted between September 2014 and November 2014. Most of the literature was obtained online from various electronic databases such as *PubMed Health*®, *Sage*® and *Science Direct*®. The initial literature search involved key-words such as; 'preservation', 'eye storage', 'cadaveric', 'bulbus/i oculi',

'dehydration' and 'embalming'. Despite using various key-word combinations, very few journal articles could be found related to the preservation of whole cadaveric eyes. Most of the scientific papers identified were related to preservation of the human cornea prior to transplantation. Thus, another literature search was conducted using different key-words such as; 'intra-ocular pressure', 'ocular volume', 'dryness' and 'model', and all the relevant articles were sought after and filtered according to relevance, date and language of publication. The bibliographies of such publications were also searched for potential referencing sources. Whenever possible, primary sources of literature were acquired.

Results & Discussion:

The intra-ocular pressure / volume relationship in a living entity

The intra-ocular pressure (IOP) is a measure of the fluid pressure inside the eye, which accumulates to achieve a balance between the inflow and the outflow of the aqueous humour as well as blood. As a result, fluid drainage can occur at the same rate at which it is produced, whilst a steady blood-flow in and out of the eye can be maintained. An average value of a steady state IOP in a living human being is approximately 15 millimetres of mercury (mmHg). There are multiple physiological factors that maintain a steady state IOP, including; aqueous humour production (F in), trabecular outflow (C trab), uveoscleral outflow (Fu) as well as venous pressure in the episcleral vessels (Pe)[6]. Under normal steady state conditions, the hydraulic equation that governs aqueous humour dynamics can be defined as follows[6-7]:

$$F\,\text{in} = F\,\text{out} = C\,\text{trab}(\text{IOP} - P\text{e}) + F\text{u} \quad (1)$$

Variations in any of these factors will cause fluctuations in the IOP as well as in the net ocular volume, particularly that contributable to the aqueous humour and blood.

Variation in the intra-ocular pressure post-mortem

Just a few seconds after death, the arterial blood pressure falls toward the mean systemic filling pressure that is defined as the mean pressure that exists in the circulatory system when the blood has had a chance to redistribute evenly to all vessels and organs, following the cessation of heart pumping. Thus, blood flow into the eye stops whilst the residual blood volume drains out of the eye. This in turn results in a net decrease in blood volume and a rapid drop in the IOP. In fact, the IOP immediately after death is typically reduced to 5 to 7 mmHg.[8-9] Aqueous dynamics, specifically fluid leakage out of the eye into either the arterial or venous systems, also plays a role in the drastic drop of the IOP post-mortem, which in turn causes flaccidity of the bulbi oculi. This is a major limitation that still needs to be overcome during preservation of the human cadaveric eye, considering there are a lot of fluids that can be injected into the bulbi oculi and which have been proven to increase the IOP to a significant amount.

The prevention of deflation of the human cadaveric eye

As can be referred to in *Table 1* below, various IOP elevation methods will now be reviewed, in relation to establishing a preservation technique which prevents deflation of the human cadaveric bulbi oculi.

(ORIGINAL) Table 1: REVIEWED METHODS OF POSSIBLE INTRAOCULAR PRESSURE ELEVATION & HYDRATION OF THE HUMAN CADAVERIC BULBI OCULI

INTRAOCULAR PRESSURE ELEVATION METHOD:	**HYDRATION METHOD:**
Administration of fluid injections to increase volume	Hyaluronic acid or carboxymethylcellulose-based solutions
Head-down tilt	Trehalose-based solutions
Induction of corneal rigidity	Hydroxypropyl-guar or hypromellose-based solutions
Cautery or clamping of the optic nerve and ophthalmic vessels	
Angle closure	
Alteration of cerebrospinal fluid pressure	

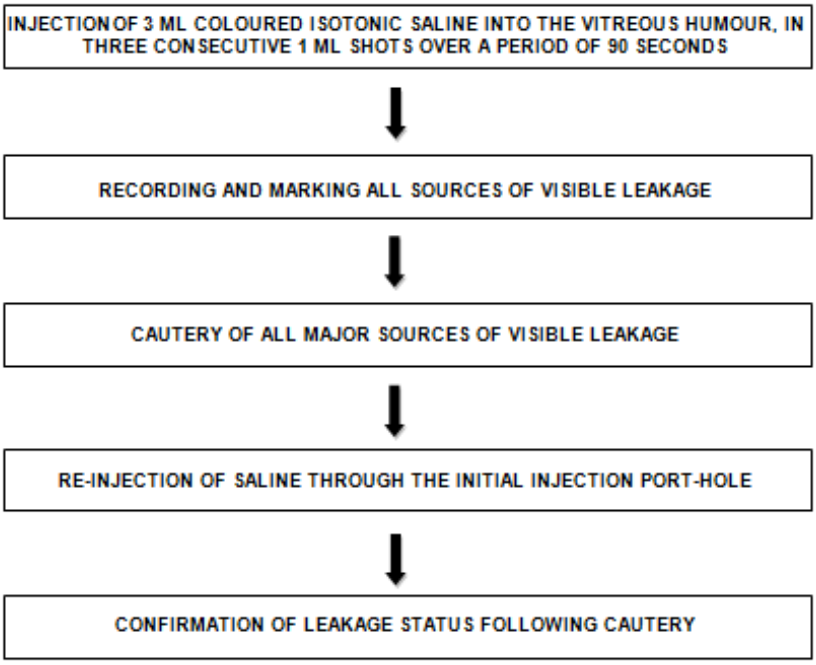
Various methods that can possibly be tested in combination post-mortem in order to prevent deflation and dehydration of the human cadaveric bulbi oculi are shown.

The administration of fluid injections to increase volume

Keeping in mind the equation discussed earlier, it can be postulated that IOP elevation will occur upon the introduction of an additional amount of fluid in a contained chamber within the human cadaveric eye, if coupled with a controlled reduction in the outflow facility. For example, it has been documented that upon the injection of 22 millilitres (ml) of expired whole blood into the retrobulbular area located behind the bulbi oculi of 10 human cadaveric orbits, the mean IOP increased rapidly from 19 mm Hg to 72 mm Hg.[10] This maneuver enforced compression of the optic nerve and vessels, and as a result, prevention of the draining away of fluid from the bulbi oculi. Unfortunately, this effect was short-lived as a result of fluids leaking out of the superior and inferior orbital fissures as well as other orbital spaces. Similar findings were obtained in another study conducted on cadaveric models.[11] Primarily, the vitreous cavity of each cadaveric globe was injected with isotonic saline to return the IOP to physiological levels. Secondly, a viscous mixture of glycerin and hydroxyethyl cellulose was also injected through the same site in order to aid the saline solutions to remain well-isolated from the injection port-site for a promising time window, considering that the initial attempts to inject only whole blood or saline to elevate the IOP led to rapid leakage of fluids out of the bulbi oculi. Another group of researchers sought to experiment on porcine cadaveric bulbi oculi, which were primarily immersed in saline up to the limbus[12]. Globes were placed on a holder to allow unrestricted volume expansion during infusion of a phosphate-buffered solution (PBS) into the posterior chamber of the eye, as this location has been proven to minimize the washout effect, which manifests as a reduction in the resistance to aqueous outflow with an increase in the volume of perfusion. The highest measurement of the IOP from baseline value after infusion of 15 ml of PBS was observed to be 24 mm Hg.

A major limitation that still needs to be addressed when injecting an amount of fluid in the human cadaveric bulbi oculi is it's leakage into the arterial or venous systems as a result of a decreased pressure brought about by death. For this reason, the authors sought to conduct preliminary testing on a set of four bovine cadaveric bulbi oculi, which were obtained less than 12 hours post-slaughter, to outline sources of major leakages. In order to prevent further dehydration of sources, specimens were immersed in isotonic saline and sealed in an air-tight container until two sets of experiments were conducted. An overview of the methodology used by the authors for this experimental study is provided in *Figure 1* below.

(ORIGINAL) Figure 1: **OVERVIEW OF THE INITIAL RESEARCH METHODS ON BOVINE EYES**



The methodology behind the authors' model study on bovine eyes with the aim of investigating deflation of cadaveric bulbi oculi via the classification and treatment of major anatomical leakage sources is shown.

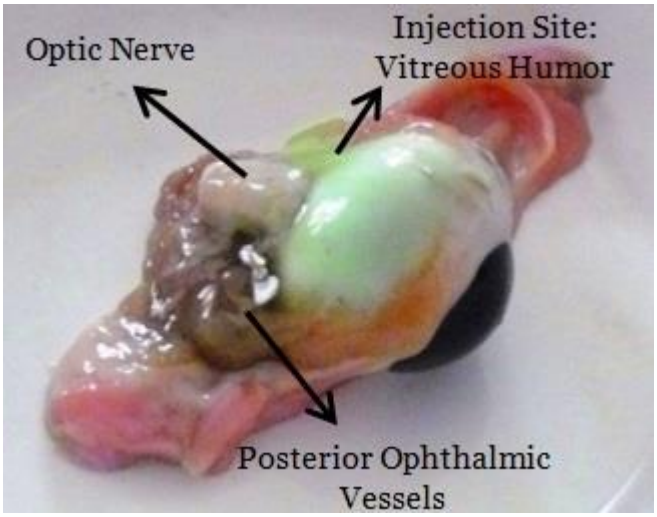
For the first test, the eyelids and ocular muscles of two bovine eyes were dissected in order to expose the bulbi oculi and the surrounding nerves and blood vessels. The second test was performed on another set of two bovine eyes, and in the latter case, muscles and surrounding tissues were kept relatively intact. A total of 3 ml of coloured isotonic saline was injected into the vitreous humour via an insulin syringe. This solution was delivered in three consecutive shots of 1ml each, whereby the injection and re-filling took place in a period of 90 seconds, in order to detect sites where fluid could potentially leak out, thereby causing deflation and dehydration of the bulbi oculi (Refer to *Figure 2* overleaf). The major leakage sources observed were marked and cauterized using a hot soldering iron. Shortly after, saline was re-injected, with the syringe left in the initial port-hole to check whether fluid is still leaking out of the previously recorded sources.

During the first test, in which the bulbi oculi had most of the ocular muscles removed, leakage occurred mostly from the posterior aspect close to the port-hole through which fluid was injected, the optic nerve as well as the ophthalmic vessels. Minimal leakage from the posterior scleral veins was noted. No leakage from the anterior aspect of the eyeball was observed. Some deflation started to be visible approximately 60 minutes post-injection. During the second test, in which the bulbi oculi had most of the ocular muscles still intact, leakage was much less when compared to the initial test. The most visible leakages were attributable to the area around the injection site, however the area around the optic nerve also showed minimal leakage over the same specific time period. No visible leakages around the tip of the optic nerve as well as the posterior scleral vessels were observed after cautery in both cases. The bovine cadaveric bulbi oculi remained significantly inflated for 90 minutes (Refer to *Figure 3* overleaf).

(ORIGINAL) Figure 2: SETTING OF PRELIMINARY TESTING ON BOVINE CADAVERIC EYES

A total of 3 ml of coloured isotonic saline was injected into the vitreous humour via an insulin syringe. This solution was delivered in three consecutive shots of 1ml each, whereby the injection and re-filling took place in a period of 90 seconds, in order to detect sites where fluid could potentially leak out, thereby causing deflation and dehydration of the bulbi oculi.

(ORIGINAL) Figure 3: OUTLINE OF THE MAJOR ANATOMICAL SOURCES OF LEAKAGE



During the first test, in which the bulbi oculi had most of the ocular muscles removed, leakage occurred mostly from the posterior aspect close to the port-hole through which fluid was injected, the optic nerve as well as the ophthalmic vessels. Minimal leakage from the posterior scleral veins was noted. No leakage from the anterior aspect of the eyeball was observed. Some deflation started to be visible approximately 60 minutes post-injection. During the second test, in which the bulbi oculi had most of the ocular muscles still intact, leakage was much less when compared to the initial test. The most visible leakages were attributable to the area around the injection site, however the area around the optic nerve has shown minimal leakage over the same specific time period. No visible leakages were observed in the tip of the optic nerve as well as the posterior scleral vessels after cautery, and the cadaveric bulbi oculi remained significantly inflated for 90 minutes.

The head-down tilt

It is to no surprise that postural changes will elicit a variation in the IOP, depending on the different durations and angles of tilt. For example, a perturbation such as the head-down tilt is thought to increase the IOP through engorgement of the ocular circulation.[13] This is due in part to the subsequent choroidal expansion against the rigid sclera tissue,[14] as a result of increased orbital venous pressue (OVP) that will in turn inhibit the ocular venous outflow.[15-16] A postulated limitation of this method would be that in order to increase the IOP by a significant amount to prevent flaccidity of the human cadaveric bulbi oculi, the whole body would need to be preserved for a while and tilted as opposed to preserving only the head and neck or the eyes of the donor cadaver. Moreover, since salt solutions used in the embalming of the whole cadaveric body are to be found in the surrounding tissues as well as blood vessels, such fluids may leak out of the orbit, reduce compression on the eye, which may cause a resultant decrease in the IOP.

Cautery or clamping of the optic nerve and ophthalmic vessels

A more permanent method that has been proven to induce elevation of the IOP when compared to the temporary effect caused by increased fluid injections is via cautery or clamping of the optic nerve and ophtalmic vessels.[17-18] Once again, this principle works by completely blocking the ocular venous outflow and closing the connection with the cerebrospinal fluid. As a result, the blood volume in the eye will increase drastically and contribute to an elevated IOP. The major limitation in applying this method on human cadaveric eyes is that the ocular venous flow would be static. However, the application of such method can be very useful in sealing any leakages of injected fluids into the arterial or venous systems and may be performed after a head-down tilt would be previously used to increase blood volume and the IOP.

Induction of corneal rigidity

From a biomechanical perspective, the volume-pressure relationship in the eye is also influenced by the geometrical and material properties of the corneoscleral shell. Thereby, the rigidity of the ocular tissue may also contribute to changes in the IOP.

In fact, in an experimental-model study,[6] the potential role of corneal stiffness in acute IOP elevation was investigated. The stiffness of fresh, non-scalded porcine corneas was altered through immersion in 1% or 4% glutaraldehdye solution for 20 minutes at room temperature. Such medium has a proven effect to increase the rigidity of the corneas by inducing collagen cross-linking.[19] The bulbi oculi were placed with the corneas facing down so no other ocular tissues will come in direct contact with the fixative. A 22-gauge needle was inserted from the limbus into the vitreous chamber and was connected to an injection syringe to be able to normalize the IOP in all eyes to 10 mm Hg, through infusion of saline at a rate of 200 µl/minute (min). A pressure sensor with a digital output was also introduced to the vitreous chamber through an 18-gauge needle, to measure the IOP changes before and after the treatment of corneas with different glutaraldehyde concentrations. This study concluded that a significantly higher IOP was observed in the bulbi oculi after the corneas were stiffened. The mean IOP increased from 15 mm Hg (+/- 1.9 mm Hg) to 19 mm Hg (+/- 2.6 mm Hg) and 24 mm Hg (+/- 1.9 mm Hg) after being immersed in 1% and 4% glutaraldehdye solutions respectively. However, this study is liable to several limitations. To start with, it was assumed by the researchers that except for corneal stiffness, all other geometrical and material properties of the eye remained essentially the same. One must keep in mind that corneal thickness may also have been altered via the fixative medium. Furthermore, the significant influence of the sclera stiffness in sudden IOP elevation was not taken

into consideration.[21] Upon taking into consideration other literature,[21-22] another limitation would be that the porcine cornea has a lower elasticity than the human cornea. Thereby, the effect of corneal stiffness could be responsible for smaller volume and IOP variations in the human cadaveric eye. Ultimately, this deficit would most probably change the structure of the cadaveric eye, including the cornea, thus altering the operative experience of a trainee for cataract surgery.

Angle closure

Angle closure may occur in anatomically predisposed eyes whereby a contact angle of 180º or more between the peripheral iris and the trabecular meshwork is established. This causes mechanical impairment of the aqueous outflow and will eventually lead to increased IOP. [23] Thus, it may be feasible to conduct further investigation via compressing a significant region of the peripheral iris to create an artificial mimicry of closed angle glaucoma in the cadaveric eye or injecting an obstructing gel-like solution. However, whether this method will have significant effects on a cadaveric eye remains to be seen.

Alteration of the cerebrospinal fluid pressure

The optic disc separates the eye from the optic nerve, which is in turn surrounded by the subarachnoid space that forms a barrier between two pressure compartments, being the IOP and the intracranial cerebrospinal fluid pressure (CSFP) [24] respectively. Thereby, it can be postulated that pressure changes in either compartment alter the pressure distribution across the optic disc, as well as the whole bulbus oculi. In fact, it has been established that the ability to control and monitor CFSP will in turn determine retrolaminar connective tissue pressure.[25]

The prevention of dehydration of the human cadaveric eye

Owing to the lack of natural tear lubrication, dehydration is another limitation that needs to be addressed in establishing an effective technique for the preservation of the human cadaveric eye, most particularly the cornea which is badly affected by dehydration and often becomes opaque. Most of the relevant literature available for review on this particular matter relates to the application on the living human cornea, however it is still considered as useful in highlighting a number of promising artificial solutions that could be investigated in light of retaining adequate moisture in the human cadaveric bulbi oculi.

Hyaluronic acid or carboxymethylcellulose-based solutions

The majority of available preparations that help in keeping living ocular tissue moist are either based on hyaluronic acid (HA) or carboxymethlycellulose (CMC). The protective mechanism of HA-based eye drops lies in the HA's natural lubricating ability to bind water and adsorb to the ocular surface.[26] On the other hand, CMC is the main compound of artificial tear drops. It is a high-molecular weight polysaccharide with muco-adhesive properties that allow prolonged residence time on the ocular surface.[27] Further experiments are needed to establish if these solutions can also prevent dehydration in the human cadaveric eye, or if a more oily barrier would be needed.

Trehalose-based solutions

Trehalose is a disaccharide that not only has anti-dehydrating properties, but also protects the ocular cell membranes and membrane proteins from deactivation or denaturation.[28] In fact, it has been concluded that the preparations based on trehalose showed the highest effectiveness in preventing cell death from desiccation, in comparison to other eye drops based on HA, CMC and polyethylene glycol.[26] This characteristic may prove to be useful in its application on the human cadaveric eye.

Hydroxypropyl-guar or hypromellose-based solutions

Products which include hydroxypropyl-guar, and one or both of the demulcents; propylene glycol and polyethylene glycol represent a new generation of ocular lubricative preparations. They are theorized to work through a biphasic mechanism of action in which they primarily bind to damaged hydrophobic areas of the ocular epithelial cells, and then restructure it by forming a protective gel matrix that provides sustained

lubrication to the eye.[29-30] In fact, it has been established that hypromellose-based gels provide a good formula to effectively reduce ocular dehydration.[31] Thus, combining them with trehalose as indicated above may in fact be the most useful solution.

Conclusion:

Several options that may be considered for devising an effective strategy for preserving the human cadaveric eye for effective use in ocular surgery training have been highlighted, whilst addressing the major limitations of deflation and dehydration. Taking into consideration the current available literature and the findings from the initial experiments conducted by the authors of this review, the use of investigation of anti-dehydrating agents, fluid injection and blocking of all exit sources may possibly turn out to be the optimal combination for preserving the human cadaveric eye. However, the fact that to date, most experimental techniques have been tested on animal models cannot be overlooked. Moreover, one also has to take into account that a number of promising solutions have been tested in isolation not in combination with each other. Thus, solutions that have been solely advised to prevent deflation by increasing the IOP may also result in dehydration of the human cadaveric eye upon their practical application. Thereby, further investigation and additional experimental research on the human as well as animal cadaveric eyes are necessary to outline a combination of solutions that show promising potential in maintaining a cadaveric eye ideal for surgery training.

References

[1] Kerckaert, I., Van Hoof, T., Pattyn, P., D'Herde, K. Endogent: Centre for Anatomy and Invasive techniques. D Med Ana. 2008;28-33.
[2] Borderie, V.M., Lopez, M., Lombet, A., Carvajal-Gonzalez, S., Cywiner, C., Laroche, L. Cryopreservation and culture of human corneal keratocytes. IOVS. 1998;39(8):1511-1519.
[3] Komuro, A., Hodge, D.O., Gores, G.J., Bourne, W.M. Cell death during corneal storage at 4ºC. IOVS. 1999;40(12):2827-2832.
[4] Lucena, D.R., Ribeiro, M.S., Messias, A., Bicas, H.E., Scott, I.U., Jorge, S.R. Comparison of corneal changes after photo-emulsification using BSS versus Lactated Ringer's irrigating solution: A prospective randomized trial. Br J Ophthalmol. 2011;95:485-489.
[5] Shuai Shi, L., Zhang, X., Shouxiang, N.L., Wang, Y., Curcio, C.A., Chen, W. Comparison of different methods of glycerol preservation for deep anterior lamellar keratoplasty eligible corneas. IOVS. 2012;53(9):5675-5685.
[6] Liu, J., He, X. Corneal stiffness affects IOP elevation during rapid volume change in the eye. IOVS. 2009;50(5):2224-2229.
[7] Kaufman, P.I. Pressure-dependent outflow. In: Ritch, R., Shields, M.B., Krupin, T. The Glaucomas. CV Mosby. 1989:219-240.
[8] Henderer, J.D., Budenz, D.L., Flynn, H.W. Jr., Schiffman, J.C., Feuer, W.J., Murray, T.G. Elevated Intraocular Pressure and Hypotony Following Silicone Oil Retinal Tamponade for Complex Retinal Detachment: Incidence and Risk Factors. Arch Ophthalmol. 1999;117(2):189–95.
[9] Kiel, J.W. (2010). The ocular circulation. San Rafael (CA): Morgan & Claypool Life Sciences.
[10] Zoumalan, C.I., Bullock, J.D., Warwar, R.E., Fuller, B., Mc Culley, T.J. Evaluation of intraocular and orbital pressure in the management of orbital haemhorrage: an experimental model. Arch Ophtalmol. 2008;126(9):1257-1260.
[11] Oester, A.E., Fowler, B.T., Fleming, J.C. Inferior orbital septum release compared to lateral canthotomy and cantholysis in the management of orbital compartment syndrome. Ophthal Plast Reconstr Surg. 2012;28(1):40-43.
[12] Morris, H.J., Tang, J., Cruz-Perez, B., Pan, X., Hart, R.T., Weber, P.A., et al., Correlation between biomechanical responses of posterior sclera and IOP elevations during micro intraocular volume change. IOVS. 2013;54:7215-7222.
[13] Lavery, W.J., Kiel, J.W. Effects of head-down tilt on episcleral venous pressure in a rabbit model." Exp Eye Res. 2013;111:88-94.
[14] Taibbi, G., Kaplowitz, K., Cromwell, R.L., Godley, B.F., Zanello, S.B., Vizzeri, G. Effects of 30-Day head-down bed rest on ocular structures and visual function in a healthy subject. Aviat Space Environ Med. 2013;84(2):148-154.
[15] Carlson, K.H., Mc Laren, J.W., Topper, J.E., Brubaker, R.F. Effect of body position on intraocular pressure and aqueous flow. IOVS. 1987;28:1346-1350.
[16] Reitsamer, H.A., Kiel, J.W. A rabbit model to study orbital venous pressure, intraocular pressure and ocular hemodynamics simultaneously. IOVS. 2002;43(12):3728-3734.
[17] Bayer, A.U., Danias, J., Brodie, S., Maag, K.P., Chen, B., Shen, F., et al., Electroretinographic abnormalities in a rat glaucoma model with chronic elevated intraocular pressure. Exp Eye Res. 2001;72(6):667-677.

[18] Diaz, F., Villena, A., Vidal, L., Moreno, M., Garcia-Campos, J., Perez de Vargas, I. Experimental model of ocular hypertension in the rat: Study of the optic nerve capillaries and action of hypotensive drugs. IOVS. 2010;51(2):946-951.
[19] Spoerl, E., Seiler, T. Techniques for stiffening the cornea. J Refract Surg. 1999;15:711-713.
[20] Sigal, I.A., Flanagan, J.G., Ethier, C.R. Factors influencing optic nerve head biomechanics. IOVS. 2005;46:4189-4199.
[21] Kotliar, K., Maier, M., Bauer, S., Feucht, N., Lohmann, C., Lanzi, I. Effect of intra-vitreal injections and volume changes on intraocular pressure: clinical results and biomechanical model. Acta Ophtalmol Scand. 2007;85:777-781.
[22] Pallikaris, I.G., Kymionis, G.D., Ginis, H.S., Kounis, G.A., Tsilimbaris, M.K. Ocular rigidity in living human eyes. IOVS. 2005;46:409-414.
[23] Chong, R.S., Sakata, L.M., Narayanaswamy, A.K., Ho, S.W., He, M., Baskaran, M., et al,. Relationship between intraocular pressure and angle configuration: An anterior segment OCT study. IOVS. 2013;54(3):1650-1654.
[24] Morgan, W.H., Chauban, B.C., Yu, D.Y., Cringle, S.J., Alder, V.A., House, P.H. Optic disc movement with variations in intraocular and cerebrospinal fluid pressure. IOVS. 2002;43:3236-3241.
[25] Morgan, W.H., Yu, D.Y., Alder, V.A. The correlation between cerebrospinal fluid pressure and retrolaminar tissue pressure. IOVS. 1998;39:1419-1428.
[26] Aragona, P., Papa, V., Micali, A., Santocono, M., Milazzo, G. Long term treatment with sodium hyaluronate-containing artificial tears reduces ocular surface damage in patients with dry eye. Br J Ophthalmol. 2002;86(2):181-184.
[27] Bator, A.H., Misiuk-Hojlo, M., Marycz, K., Grzesiak, J. Trehalose-based eye drops preserve viability and functionality of cultured human cornal epithelial cells during desiccation. BMed R Int. 2014;8:110-119.
[28] Elbein, A.D., Pan, Y.T., Pastuszak, I., Carroll, D. New insights on Trehalose: A multifunctional molecule. *Glycobio*. 2013;13(4):17-27.
[29] Christensen, M.T., Cohen, S., Rinehart, J. Clinical evaluation of an HP-guar gellable lubricant eye drop for the relief of dryness of the eye. *Curr Eye Res*. 2004;28(1):55-62.
[30] Bernelli, U. Systane® lubricant eye drops in the management of ocular dryness. *Clin Ophthalmol.* 2011;5:783-790.
[31] Tauber, J. Efficacy, tolerability and comfort of a 0.3% hypromellose gel ophthalmic lubricant in the treatment of patients with moderate to severe dry eye syndrome. *Curr Med Res Opin.* 2007;23(11):2629-2636.

A Brief Author Biography:

Georgiana Farrugia, BSc (Hons). – Ms. Georgiana Farrugia is a diagnostic radiographer, and is currently reading for her Doctorate in Medicine & Surgery at the University of Malta. Her current research interests are cardiology, respiratory medicine and radiology.

Jean Calleja Agius, MD, Ph.D, MRCOG, MRCP, MSc. – Prof. Jean Calleja Agius graduated as a Doctor of Medicine & Surgery in 1999. She further specialized in Obstetrics & Gynaecology, both locally and at the University College London Hospital. She obtained her memberships of the Royal College of Obstetricians and Gynaecologists and of the Royal College of Physicians of Ireland, on her first attempt in 2005. She was appointed as Assistant Lecturer at the University of Malta in 2000, and since then has been teaching medical students, post-graduate trainees and para-medic professionals. She has obtained a distinction in her MSc in Clinical Embryology at the University of Leeds. She is currently the Head of the Department of Anatomy, of the Faculty of Medicine & Surgery at the University of Malta. She has numerous peer-reviewed articles published in international high impact journals. She obtained her PhD at the University College London in 2012. The current research interests of Prof. Calleja Agius are embryology and reproductive medicine, particularly recurrent miscarriages and infertility.

Pierre Schembri Wismayer, MD, Ph.D. – Dr. Schembri Wismayer is an associate professor at the Department of Anatomy, of the Faculty of Medicine & Surgery at the University of Malta. He read for his undergraduate medical degree at the University of Malta, and after two years of housemanship, he did some voluntary medical work in Kenya, and then left to pursue his Ph.D in molecular oncology at the Beatson Institute for Cancer Research in affiliation with Glasgow University. The current researh interests of Dr. Schembri Wismayer are cancer stem cells, leukemia differentiation, natural product bioactivity analysis and transcriptional regulation.

