Research Article

The Elnady Technique: An Innovative, New Method for Tissue Preservation

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Summary

At the Faculty of Veterinary Medicine, Cairo University, there is an increasing number of students but a limited availability of animal cadavers for dissection, and student exposure to formalin is a known hazard. In order to address these challenges, a new method for tissue preservation was developed, the "Elnady Technique." This method is a modified form of plastination, where the chemicals used are not patented, are inexpensive and locally available, and the process is performed at room temperature. The produced specimens are realistic, durable, have no offensive odor, and are dry, soft and flexible. They can be used to replace the use of animals killed for teaching basic anatomy, embryology, pathology, parasitology and forensic medicine. They have great potential to support training in clinical skills and surgery, including for clinical examination, endoscopy, surgical sutures, and obstetrics simulation.

Keywords: veterinary anatomy, alternatives, plastination, preservation

1 Introduction

Putrefaction is a feature of all biological tissues, and various methods of tissue preservation have been developed over time. Ancient Egyptian embalmers used natron for human cadaver mummification, and centuries later other techniques using embalming solutions such as formalin were developed. The process of fixation using formalin is well known (Brenner, 2014) and can be performed in several ways, including injection, infiltration, immersion or diffusion (Ostrom, 1987). The method chosen for fixation depends on the target specimen.

The process of plastination was developed by Gunther von Hagens in 1977 and has been used successfully all over the world. However, establishing a plastination laboratory is expensive, and the chemicals are patented and often are not available. The "Elnady Technique" is simple, inexpensive and uses non-patented chemicals. Although the process is patented for commercial use, it is shared openly in this paper. The specimens that are produced using this method are realistic, durable, and more flexible than plastinated specimens, besides being clean and having no offensive odor. They can have a great impact by enhancing education and training. Use of the specimens can achieve replacement of animals that are killed for dissection, used in experiments, or used for other instrumental purposes.

2 Animal, materials and methods

This work was performed at the Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Cairo University, Egypt. The author followed the guidance and definitions described in the *InterNICHE Policy on the Use of Animals and Alternatives in Education and Training* (Martinsen and Jukes, 2007). The origin of each animal and the reason for death or euthanasia were considered so that the animal cadavers were "ethically sourced" or from an "acceptable other source", according to the Policy (Jukes, 2014). Most of the animals (camel, buffalo, ox, horse, donkey, sheep, goat, dog, cat and chicken) were euthanized in the clinic due to serious non-recoverable injury or terminal illness. Others were naturally aborted fetuses or were found cadavers. They comprised stray animals, working animals, and patients from the surgery clinic at the faculty hospital donated with the owner's consent.

Proper selection of potential individual animal cadavers and specimens is important from both an ethical and practical perspective. Correct selection ensures suitability for their potential use in practical classes and avoids wasting time, effort and money in preserving poor specimens. Slim, non-castrated animals are preferred, as castrated ones typically have excess fat. Animals that have died from severe septicemia or infectious diseases are not recommended for preservation to repre-

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sent healthy structures; however, rare pathological specimens may be preserved.

To ensure humane euthanasia of the patients and to prepare for preservation, the animals were intravenously injected with a tranquilizer, xylazine, and anesthetic solution, thiopental sodium. On being unconscious, in full surgical anesthesia, the common carotid artery was dissected dorsolateral to the trachea, then cut. The animal was allowed to bleed until death.

The steps of the Elnady Technique are described below:

Step one: Fixation with formalin
Tissue fixation proceeds in a way similar to that used in plastination. The process has been covered in detail in previous articles about plastination (Ostrom, 1987) and some points will be highlighted.

The method chosen for tissue fixation using formalin depends on the target specimen. Our preferred method is euthanasia of the animal along with bleeding, then cannulation and injection of 10% formalin into the common carotid artery. The amount of formalin injected depends on the size of the animal. The embalming fluid is introduced either by syringe with hand pressure or (better for large animals) by gravity-feed from a container held 3 to 6 feet (0.9 - 1.8 m) above the specimen, depending on the pressure needed. Usually, when there is resistance for injection of the embalming solution, this is an indication that sufficient solution has been injected. For example, a medium-sized horse requires 70-80 liters of the embalming solution while about 1000 ml are needed for a cat.

The cadavers are then left in the laboratory at room temperature for the tissues to become fully saturated with formalin. This takes one to two weeks, for small and large animals, respectively. Formalin penetrates faster than many other fixatives (about 6 mm per 12 hours). After injection, tissues should become somewhat bloated. Ears are erected, limbs become stiff and the tongue should be stiff, pale and whitish. After this period, the cadaver is dissected to provide a specimen for future use by students and trainees. The specimen can be a limb, organ or tissue that illustrates structure sufficiently for the purpose of a practical class such as within anatomy, embryology or surgery.

Specimens obtained from animals that have been dead for some time are cannulated through the major arteries, injected with 20% formalin, and then immersed in a 5% formalin bath for about 3 weeks. This low formalin concentration bath allows the formalin to reach the tissues of superficial and deep structures. Higher concentrations would only saturate the tissues of superficial structures, and the tissues of deep structures could putrefy.

For those specimens where cannulation is not possible, for example due to blood clotting, and in all large specimens (such as limbs of horses and donkeys), additional bone drilling and further dissection (as described in Step two below) are required. The specimens are then immersed in a 5% formalin bath for around 3 weeks.

For temporary storage any specimen is immersed in a bath of 5% formalin, for example in case specimens are to be collected to be processed together or if a dissection needs to be continued for several days or weeks.

Step two: Dye injection, muscle dissection and bone drilling
For specimens where cannulation is possible, colored latex or epoxy can now be injected into vessels or cavities for better visualization of structures if required.

For all large specimens, the thick muscle tissue of limbs of large animals should be further dissected. The skin and superficial fascia are incised and reflected, and then the deep fascia is split to allow muscles to separate from each other in part or in full. This ensures that the acetone and glycerin used respectively within the next steps of dehydration and impregnation will penetrate the entire specimen.

It is also important to work on bones. Small holes are drilled into the marrow cavity of long bones in less visible areas to enhance defatting and to prevent greasy specimens. In some joint specimens where bones are cut, bone marrow is curetted via the cross sectional openings.

At this stage, hollow organs like heart, stomach and intestine should be cleaned of blood and ingesta by washing under running water. They can then be stuffed, if desired, with industrial polyester fibers used in upholstery to fill the cavities.

Step three: Dehydration with acetone
The process of dehydration has been addressed in previous articles about plastination (Klaus and Dubravka, 1988; Henry, 1998). Using the Elnady Technique, the full process of dehydration is performed at room temperature. The limb, organ or tissue is then washed with water, and immersed in a pure (100%) acetone bath and left for one week. Afterwards, the concentration of acetone is measured using a hydrometer, and the specimen is transferred to a new bath of 100% concentration and left for another week. The concentration is intermittently re-measured, and when it remains constant at 98-99%, the specimens are considered dehydrated.

Step four: Impregnation in glycerin
The dehydrated specimens are gently pressed by hand and allowed to drain of acetone through a sieve. They are then fully immersed in a glycerin bath for one to two weeks according to the type and size of the tissue. Small specimens, hollow organs and thin walled tissue of any size require about one week for complete impregnation.

Step five: Curing with cornstarch
The specimens are taken out of the glycerin, allowed to drain, then wiped down thoroughly using tissue paper. They are then transferred into cloth bags that are two or three times the size of the specimens and cornstarch powder is added. The bags are tightly ligated, then rubbed with cornstarch powder from outside. Small specimens may instead be immersed in containers filled with cornstarch powder for one to three days. During this time it is recommended to intermittently turn and massage the specimens within the bags. Cornstarch powder that is saturated with glycerin begins to clump and should be replaced with new powder. Finally, after about one week, when the starch no longer clumps, the specimens are carefully taken out of the bags. Residue of cornstarch on the specimens can be removed using a soft brush or be blown off with air.

If the specimen is still oozing glycerin, it is returned to the cloth bag and the process is repeated until the cornstarch no longer clumps. If necessary, the specimen can instead be rubbed directly with cornstarch and left for one or more weeks in the open air resting on flat cardboard to help absorb glycerin.

When no more glycerin is visible, the specimen is cured. Acrylic or fabric colors can then be used to paint vessels or other structures such as the pericardium or muscles. The finished specimen is stored in a hygienic location, like a museum, or in a sealed container or plastic bag.

3 Results

A variety of specimens were developed, representing different body systems from different animal species. The preserved specimens are realistic, durable and, unlike after conventional plastination, are soft and flexible. They are clean and have no offensive odor.

Fig. 1: Images of hollow viscera preserved with the Elnady Technique
(a) Heart of a horse with the pericardium reflected; (b) longitudinal section through the heart of a horse; (c) stomach of a horse; (d) longitudinal section through the stomach; interior of the stomach of an ox: (e) reticulum, (f) omasum and (g) rumen; (h) intestine of a horse.

We used acetone at room temperature for dehydration and the specimens described below have minimal shrinkage and good colors. The only organ that showed severe shrinkage was the whole testis of the horse; however, when the fibrous capsule (tunica albuginea) was sectioned before impregnation, there was little shrinkage.

The specimens are from the following categories:

Hollow viscera
The preserved specimens that have been developed include the heart, with its pericardium, of four horses, one donkey and one buffalo calf. The internal features of the heart are clearly demonstrated. The stomach, small and large intestine of horses and donkeys were also prepared. The external and internal anatomical features are clearly shown and can be further dissected at a future date if chosen. The uterus, with ovaries, of a mare, she-camel and cow were preserved in addition to the complete urogenital system of a mare. Examples are shown in Figure 1.

Viscera in situ
The abdominal viscera of three horses and the thoracic and abdominal viscera of two newborn goats, a cat, dog, rabbit, frog, two guinea pigs and a chicken were preserved *in situ*. The viscera comprised the heart, lungs, diaphragm, spleen, liver, stomach,

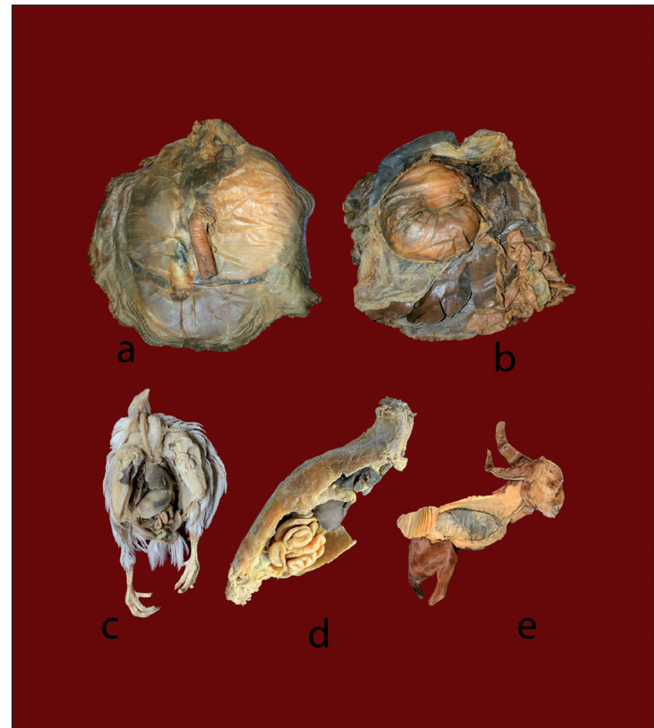

Fig. 2: Images of viscera *in situ* preserved with the Elnady Technique
(a) Diaphragmatic and (b) visceral surface of the abdominal viscera of a horse; (c) viscera of a chicken; (d) viscera of a cat; (e) viscera of a newborn goat (ribs are reflected).

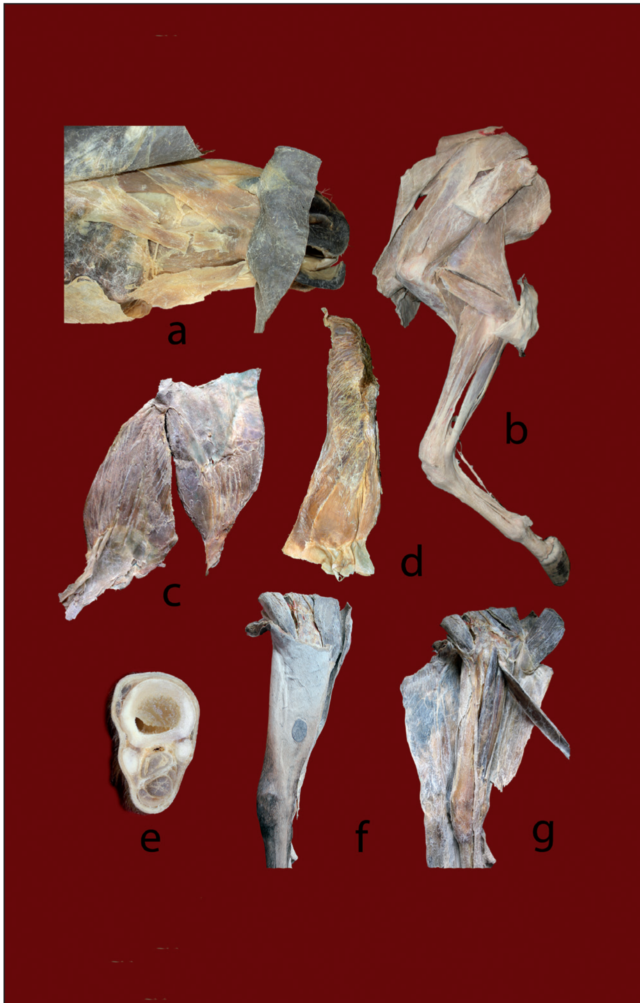

Fig. 3: Images of musculoskeletal specimens preserved with the Elnady Technique
(a) Face of a horse with skin reflected; (b) forelimb of a donkey; (c) superficial gluteal and tensor fasciae latae muscles; (d) biceps femoris muscle; (e) cross section in the distal limb of a horse pelvic limb; (f) forearm of a horse covered with skin; (g) forearm with the skin reflected showing muscles.

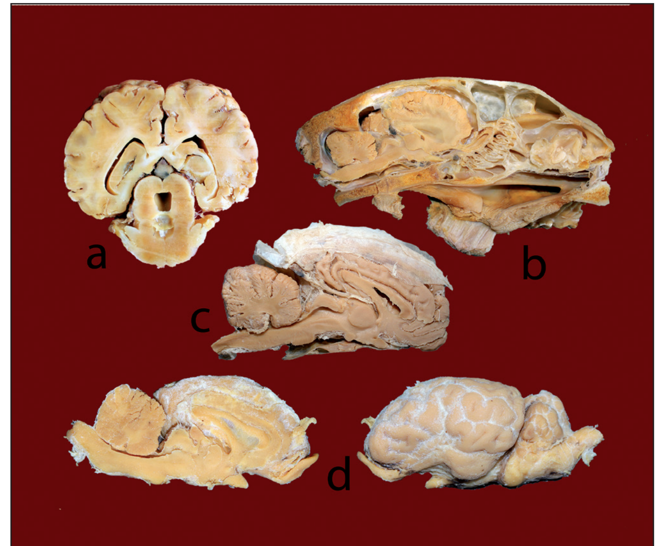

Fig. 4: Images of brain specimens preserved with the Elnady Technique
(a) Cross section of a donkey brain (b) Sagittal section of a horse head showing brain *in situ* (c) Sagittal section of a horse brain (d) Sagittal section of a sheep brain medial view (left) and lateral view (right).

kidneys and adrenals together with their accompanying serous membranes: the pleura, pericardium and peritoneum. The neurovascular supply of the viscera is clearly visible, especially if vessels were previously injected with colored latex. In some cases, acrylic colors were used to paint vessels or other structures such as the pericardium or muscles after complete curing. Specimens representing stomach compartments of the ox and sheep were developed, including the rumen, reticulum, omasum and abomasum. See examples in Figure 2.

Musculoskeletal preparations
Some muscles of the horse were isolated and preserved, including muscles of the forelimb and pelvic limb with their attached tendons of origin and insertion. The whole forelimb of a horse, donkey and dog were also preserved. In the case of the horse, for convenience in storage and transport, the limb was cut into three segments that could be mounted together. Layered muscles of the face of the horse were preserved with the covering skin and all associated blood vessels, nerves and other anatomical structures. Ligamentous preparations of joints, such as the stifle joint of a horse and dog, and the reciprocal apparatus of the horse were developed. Cross sections of a horse distal limb, and cross and sagittal sections of the head of a horse, sheep and goat were also made and preserved. Some fetuses were preserved as a whole, including the fetus of a horse, buffalo, goat twins, and camel. A coronal section of the buffalo fetus and sagittal section of a camel fetus were prepared. Exemplars of musculoskeletal preparations are shown in Figure 3.

Brain specimens
The brain of three horses, two donkeys, two sheep and one goat was preserved. Some brain specimens were cut into various planes of section. Others were kept as a whole with both eyes connected to the brain. The brain was also preserved *in situ* in sagittal sections of the head. The cranial dura mater of a horse and a donkey was carefully preserved. See Figure 4 for examples.

Miscellaneous specimens
Other specimens included a whole bat, placenta of a buffalo, penis and prepuce of a donkey, male genital system of a horse, hoof wall and the sensitive laminae of horses and donkeys, and eyes from different animal species including dog, horse and cow. Some parasites, such as the horse bot fly larvae, were preserved within the host and separately, to be used within parasitology practical classes. Some miscellaneous specimens are depicted in Figure 5.

Fig. 5: Images of miscellaneous specimens preserved with the Elnady Technique

(a) Sagittal section of the horse head; (b) sagittal section of head of a donkey fetus, some structures were colored in one half; (c) sagittal section of the penis and prepuce of a donkey; (d) eyeball of a horse opened; (e) sensitive laminae of a horse foot; (f) bat; (g) hoof wall and frog of a horse; (h) goat fetus; (i) placenta of a buffalo.

4 Discussion

Although there are several plastination laboratories in Egypt, the cost of setting up an own small unit is still beyond the limits of many colleges. This is especially true for production of medium and large sized specimens (Baptista et al., 1992). Another challenge is that the main chemicals used in plastination are patented and must be imported from abroad. The chemicals used for the Elnady Technique are known, inexpensive and locally available. The Elnady Technique for tissue preservation is registered for a patent for commercial use in Egypt (number 2051/2014).

In the dehydration process, acetone is commonly used in plastination either at -25°C (Klaus and Dubravka, 1988) or at room temperature (Zheng et al., 1998). In the Elnady Technique, the use of acetone at room temperature successfully produced good specimens. In addition, its use at room temperature minimizes the risk of explosion and obviates the need for explosion-proof freezers, reducing overall costs.

Impregnation is the main step after dehydration. Glycerin jelly has been used in medical and biological science museums to embed zoology and botany specimens in sealed glass jars (http://www.aciscience.org/docs/uses_of_glycerine.pdf). In addition, glycerin has been added to many embalming solution formulas like that used for preserving Rosalia Lombardo (Piombino-Mascali et al., 2009). Also, glycerin has been used to develop transparent or semitransparent specimens (An et al., 2012; Li et al., 2012). Moreover, glycerin has been used for preservation of isolated organs (Carvalho et al., 2013; Gigek et al., 2009; Silva et al., 2008; Cury et al., 2013).

In the Elnady Technique, glycerin is used because it has four important properties that support tissue preservation: it is non-toxic, has plasticizing and hygroscopic effects, and has a high penetrating power. In many biodegradable packaging materials glycerol is added as a plasticizer to decrease the brittleness of the product and create the desired flexibility (Nashed et al., 2003). The power of glycerin to strongly penetrate deeply into the tissues eliminates the need for vacuum pumps that are used in plastination to saturate the tissues with the polymer.

The final stage is curing. Heat, ultraviolet light and gas curing using silicone-6 (S-6) are methods used in plastination while in the Elnady Technique cornstarch is applied via cloth bags. The cornstarch binds glycerin, allowing the surface of the tissues to become dry and loose its stickiness.

The produced specimens are realistic, durable, have no offensive odor, and are dry, soft and flexible. They also exhibit beautiful colors that are very close to the original unpreserved specimens. Stored hygienically, they will last without deterioration for a long period. Those described above are in excellent condition over one year after production.

The Elnady Technique can be used to help teach basic anatomy, embryology, pathology, parasitology and forensic medicine. The softness of the cadavers, organs and tissue developed using the method allow for dissection of the specimens by students in practical anatomy classes and post mortem examinations in pathology. The softness and flexibility are also essential for efficient demonstration of some basic anatomy and biomechanics concepts. These include the patellar locking mechanism, the reciprocal apparatus of the equine pelvic limb, and demonstration of the shock absorbing mechanism achieved via the elasticity of the hoof collateral cartilages. Rare pathological specimens can also be preserved without risk of infection, as the microorganisms have been exposed to formalin and acetone dehydration during the process of preservation.

The specimens have great potential to support training in clinical skills and surgery, including for clinical examination, endoscopy, surgical sutures, abdominal laparoscopy, obstetrics simulation, and orchiectomy and ovariohysterectomy. The flexibility of the preserved tissue facilitates handling within specific clinical procedures, such as endoscopy training on introducing a Foley catheter into the ostia of the guttural pouch (Elnady et al., 2015).

Finally, production of sectioned preserved specimens can play an important role in interpretation of recent imaging mo-

dalities including magnetic resonance imaging (MRI), computed tomography (CT) and ultrasound (Latorre et al., 1998, 2001; Lawrence and Gunther, 1988).

5 Conclusion

The Elnady Technique is an innovative, new and inexpensive process for tissue preservation. All steps run at room temperature. It uses locally available chemicals: formalin for fixation, acetone for dehydration, glycerin for impregnation and cornstarch for curing. The developed specimens are realistic, durable, have no offensive odor, and are dry, soft and flexible. They are of great help to students and teachers where they can provide new opportunities for hands-on experience in the practical classes of a wide range of disciplines. The technique has been well received at the Faculty of Veterinary Medicine at Cairo University, and has great potential for enhancing education and training and replacing the harmful use of animals at other universities.

References

An, X., Yue, B., Lee, J. H. et al. (2012). Arterial anatomy of the gracilis muscle as determined by latex injection and glycerin transparency. *Clin Anat 25*, 231-234. http://dx.doi.org/10.1002/ca.21217

Baptista, C. A., Bellm, P., Plagge, M. S. et al. (1992). The use of explosion proof freezers in plastination: Are they really necessary? *J Int Soc Plastination 6*, 34-37.

Brenner, E. (2014). Human body preservation – old and new techniques. *J Anat 224*, 316-344. http://dx.doi.org/10.1111/joa.12160

Carvalho, Y. K., Zavarize, K. C., Medeiros, L. d. S. et al. (2013). Evaluation of the glycerin from biodiesel production in the preservation of anatomical parts. *Pesq Vet Bras 33*, 115-118. http://dx.doi.org/10.1590/S0100-736X2013000100021

Cury, F. S., Censoni, J. B. and Ambrósio, C. E. (2013). (Técnicas anatômicas no ensino da prática de anatomia animal). *Pesq Vet Bras 33*, 688-696. http://dx.doi.org/10.1590/S0100-736X2013000500022

Elnady, F. A., Sheta, E., Khalifa, A. K. et al. (2015). Training of upper respiratory endoscopy in the horse using preserved head and neck. *ALTEX 32*, 384-387. http://dx.doi.org/10.14573/altex.1505111

Gigek, T., Oliveira, J. E. M., Neto, A. C. A. et al. (2009). (Estudo Analítico da Técnica de Glicerinação Empregada para Conservação de Peças Anatômicas de Bovinos). *Anais V Simpósio de Ciências da Unesp Dracena*, SP. 1-3.

Jukes, N. (2014). Ethical animal use in education and training: From clinical rotations to ethically sourced cadavers. *ATLA 42*, 9-12.

Henry, R. W. (1998). Principles of plastination – dehydration of specimens. *J Int Soc Plastination 10*, 27-30.

Klaus, T. and Dubravka, I. (1988). Dehydration of macroscopic specimens by freeze substitution in acetone. *J Int Soc Plastination 2*, 2-12.

Latorre, R., Vazquez, J., Gil, F. et al. (1998). Macroscopic interpretation of horse head sectional anatomy using plastinated S-10 sections. *J Int Soc Plastination 13*, 39.

Latorre, R., Vazquez, J. M., Gil, F. et al. (2001). Teaching anatomy of the distal equine thoracic limb with plastinated slices. *Int Soc Plastination 16*, 23-30.

Lawrence, M. and von Gunther, H. (1988). The diagnostic imaging characteristics of plastinated anatomical specimens. *J Int Soc Plastination 2*, 24-38.

Li, J., Shi, S., Zhang, X. et al. (2012). Comparison of different methods of glycerol preservation for deep anterior lamellar keratoplasty eligible corneas. *Invest Ophthalmol Vis Sci 53*, 5675-5685. http://dx.doi.org/10.1167/iovs.12-9936

Martinsen, S. and Jukes, N. (2008). From policy to practice: Illustrating the viability of full replacement. *AATEX 14, Spec. Issue,* 249-252.

Nashed, G., Rutgers, R. P. G. and Sopade, P. A. (2003). The plasticisation effect of glycerol and water on the gelatinisation of wheat starch. *Starch-Starke 55*, 131-137. http://dx.doi.org/10.1002/star.200390027

Ostrom, K. (1987). Fixation of tissue for plastination: General principles. *J Int Soc Plastination 1*, 3-11.

Piombino-Mascali, D., Aufderheide, A. C., Johnson-Williams, M. et al. (2009). The Salafia method rediscovered. *Virchows Arch 454*, 355-357. http://dx.doi.org/10.1007/s00428-009-0738-6

Silva, E. M., Dias, G., Tavares, M. et al. (2008). Study of glycerination method used for conservation of anatomic parts – Experience of Anatomy discipline of the Morphology Department / UniFOA. Cadernos UniFOA, Volta Redonda, RJ, Special Edition Graduate 01. 66-69.

Zheng, T., Liu, J. and Zhu, K. (1998). Plastination at room temperature. *J Int Soc Plastination 13*, 21.

Conflict of interest

The Elnady Technique for tissue preservation is registered for a patent for commercial use in Egypt (number 2051/2014).

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