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(Short Communication)

Comparative Study of Dehydration Methods in Plastination of Piglet and Kid Cadavers as Anatomy Specimens

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Abstract

Background: In the field of Veterinary anatomy in the Philippines, the traditional method of using embalmed specimens in teaching anatomy is still widely used, as plastination requires intensive labor, high costs, and a lengthy process. Given current research exploring alternative plastination techniques that use locally available resources, this study compared acetone and ethyl alcohol as dehydration solutions for room-temperature and passive plastination. **Methodology:** Using the modified Elnady technique of plastination, one (1) piglet and one (1) kid were subjected to acetone as a dehydration solution (Treatment 1), while one (1) piglet and one (1) kid were subjected to ethyl alcohol dehydration (Treatment 2). The plastination procedure lasted for approximately 25 weeks. The weight and color of the specimens were recorded pre- and post-plastination and were analyzed descriptively. **Results:** Preliminary results show that there is a 29.21% (Kid) and 23.81% (Piglet) decrease in the weight of specimens dehydrated with acetone (Treatment 1), while 35.19% (Kid) and 33.85% (Piglet) decrease in the weight of specimens dehydrated with ethyl alcohol series. The color of the organs was slightly darker after the impregnation, but lightened after the curing process. Although a significant weight reduction was noted, the morphological features of the specimens were well-preserved and identifiable; also, the specimens exhibited flexibility, firmness, no odor, and a natural appearance, with some color variations observed between the two dehydration methods. **Conclusion:** The preliminary results suggest that ethyl alcohol can serve as an alternative dehydration solution, yielding preserved specimens that can serve as useful models as a supplement to dissection studies.

Keywords

Alternative plastination, Ethyl alcohol dehydration, Room-temperature plastination, Elnady technique

1. Introduction

Anatomy is an essential course in veterinary medicine, the backbone of veterinary medical education [1]. The dissection of animal cadavers is commonly studied in this subject. Dissection is a crucial learning experience that enhances manual dexterity, complements theoretical knowledge with practical application, highlights individual anatomical differences, fosters collaboration, and encourages personal reflection [2].

The coronavirus disease 2019 (COVID-19) has had a profound impact on anatomy education [3]. During the pandemic, students had no access to cadavers, which have been the primary way to learn anatomy since the 17th century. The outbreak prompted anatomy educators to revisit all possible teaching methods to develop innovations. A research study proposed using various techniques for anatomy education: living and radiological anatomy teaching and computer-

based learning, including virtual reality (VR), augmented reality (AR), and three-dimensional printing (3DP) digital models [4]. Plastinated specimens have been used to expand the quality of teaching and learning anatomy [5,6]. Further, many studies have concluded that students are motivated and interested in using such technologies as augmented reality (AR) and virtual reality (VR) [7,8]. Despite the availability of digital models and computer-based learning, not all students can access such resources, while plastinated specimens may offer these students an alternative way to study structures without the need to carry embalmed specimens for independent study.

Plastination is a technique developed for the preservation of biological specimens as dry and odorless, which can facilitate 'out of the dissection hall' teaching and overcome the disadvantage of formalin-fixed specimens, which are wet and irritating to the eyes and the airways. It has four steps: fixation, dehydration, forceful impregnation, and tissue curing. Compared to organs preserved in formalin, plastinated organs exhibit superior morphology and structure preservation, enabling the demonstration of intricate anatomical structures. Although not a replacement for traditional dissections, plastination provides an additional learning tool for long-term preservation and for teaching complex anatomy [9]. However, standard plastination is an expensive technique because of the equipment and chemical materials required; the materials and equipment can be difficult to obtain in some areas [10].

Hence, several studies have been conducted to develop a cost-effective, non-patented, less complicated plastination technique that utilizes locally available chemicals, and the process is performed at room temperature [10,11,13-18]. Exploration on the use of other dehydration solutions was also conducted by utilizing isopropyl alcohol instead of acetone for dehydration following the standard plastination technique [11-13]. These studies utilized isopropyl alcohol and absolute alcohol, while this current study explores the use of ethyl alcohol in increasing concentrations for dehydration. Modification of the impregnation solution was also conducted, like the use of xylene with silicone [14] for lightweight plastination and the use of alkyd resin [10]. Plastinates produced were lightweight specimens that are easy to carry and can easily be used for teaching, odorless, completely biosafe, robust, dry, flexible, durable, natural in color and texture, and can be used for a long time without decay [10,11,13-18].

Various modifications were also explored, like the use of room temperature plastination [15], the passive plastination method [16,17,18], and a combination of forced and passive impregnation [15,19] during the embedding process. The Elnady Technique (Elnady, 2016) combines the utilization of room temperature and passive plastination and uses locally inexpensive, non-patented chemicals. The study resulted in specimens that are realistic, durable, dry, soft, flexible, have no odor, and maintain good coloration. This research paved the way for developing countries with limited resources to create plastinated specimens that are easily developed using local resources and processed at room temperature.

Plastination in veterinary medicine in the Philippines is limited due to its intensive labor, high cost, and long duration of the process when compared with the utilization of traditional preservation methods. Therefore, the widespread use of the traditional method of embalming specimens in anatomy teaching persists. However, a multitude of studies have been undertaken to assess the risks associated with the use of formaldehyde as a fixative and individuals' exposure levels in laboratory settings. Belmonte *et al.* (2012) conducted a study that found that formaldehyde concentrations in laboratories exceeded the 0.75 ppm threshold, with a general trend of increasing over time. Health risks to laboratory personnel and students may arise when formaldehyde concentrations in the air exceed the established threshold [20]. This limits the appreciation of students for animal specimens during dissection due to the unpleasant and detrimental effects of formalin exposure on students. Therefore, this research was conducted to compare and evaluate the use of room temperature plastination with the use of acetone and increasing ethyl alcohol concentration for dehydration in the plastination of whole body plastinates of kids and piglets. This whole-body plastinate can offer relational studies among organs relative to their position in the body.

2. Methodology

Specimens

Specimens used in the study were preserved specimens. The two piglets, approximately three months of age, died naturally, and the two kids were stillborn from a doe that died from an accident. The specimens were collected and fixed with 10% formalin solution and displayed as neonate specimens at the anatomy laboratory. The piglets were fixed for eight months, while the kids were fixed for 26 months before they were subjected to washing for 72 hours (24 hours in running water and 48 hours soaked in tap water). After washing, the specimens were pre-dissected. The left and right lateral sides were dissected, and the limbs were separated to expose the thoracic and abdominal cavities, allowing exposure of the visceral organs of the piglets in Treatments 1 and 2, respectively. While a transverse incision between the thoracic and abdominal regions of the kids in Treatments 1 and 2 was conducted to expose the visceral organs. The purpose of the transection is to prepare the specimens in their pre-dissected form before subjecting them to the plastination procedure. After which, the specimens were weighed using a digital weighing scale.

Plastination Procedure

The plastination procedure was modified from the Elnady technique (Elnady, 2016). The Dye injection, muscle dissection, and bone drilling were not followed in the procedure. The dehydration protocol was modified by utilizing an increasing ethyl alcohol concentration as a dehydration solution in comparison with the standard acetone solution for dehydration. Since the specimens used in this preliminary study were fixed and stored over different time periods, the plastination procedure timeframe excluded the fixation time of the specimens used. Both the dehydration and impregnation procedure was conducted at room temperature and a passive process. Purposive distribution of the piglets and kids into two treatment groups was conducted before subjecting the specimens to the dehydration process.

Dehydration

The dehydration process was performed at room temperature. Treatment 1 (one piglet and one kid) specimens were submerged in three changes of a 100% acetone bath for one month each bath. In Treatment 2, the specimens (one piglet and one kid) were submerged in increasing concentrations of ethyl alcohol (80% and 90%) for one month each, with two changes to absolute ethyl alcohol occurring every two weeks during the third month. The entire dehydration process lasted for three months. Although Elnady (2016) dehydrated the specimens for a duration of three weeks, the researcher opted to prolong the duration of dehydration since the specimens were whole-body preparations, allowing penetration of the solution in deeper parts of tissues. The concentrations of acetone and ethyl alcohol used were measured using a hydrometer. The concentration of acetone and ethyl alcohol is re-measured before changing it to a new solution. When the concentration remains at 98-99%, the specimens are considered dehydrated, which was achieved after three changes in acetone and four changes in ethyl alcohol. This whole process of dehydrating the specimens was done in stainless steel airtight containers. The piglet and the kid were placed in the same container with 22 liters of acetone and 20 liters of ethyl alcohol in each change.

Glycerin Impregnation

The specimens were removed from the dehydration solutions and were allowed to drain excess acetone/alcohol in a draining tray. After draining excess acetone/alcohol, the specimens from Treatment 1 and Treatment 2 were submerged in glycerin in a plastic container with a lid. The volume ratio was five times the size of the specimen [16], and the total volume used for Treatment 1 was 22 liters, and 20 liters for Treatment 2. Passive impregnation allows glycerin to penetrate the specimen slowly, replacing acetone and ethyl alcohol in the specimens. The impregnation process was done for 12 weeks on both treatment groups. After impregnation, the specimens from Treatment 1 and 2 were removed from the glycerin bath and were drained for five days in a draining rack.

Curing with Corn Starch

Once the impregnation was completed, the specimens were cleaned of excess glycerin with adsorbent paper. After which, specimens were completely covered with cornstarch powder in plastic containers with a cover for 10 days. During

the curing process, the specimens were turned once daily. The cornstarch was replaced when it was saturated with glycerin, as shown by the clumping on most of the specimens' surfaces. Two changes of cornstarch were done during the 10-day curing process. The specimens were taken out of the container, and cornstarch residues were removed with the use of a paintbrush.

Data Collection and Analysis

The weight of specimens after washing was obtained as pre-plastination weight and was then measured after curing for its post-plastination weight using a digital weighing scale. Data were presented in frequencies and percentages and analyzed descriptively. The percentage reduction can be calculated using the formula:

$$\%\ reduction = \frac{Pre\text{-}plastination - Post\text{-}plastionation}{Pre\ \ plastination} x100$$

The researcher noted data on color, texture, flexibility, and morphological changes through keen observations and analyzed them descriptively. Flexibility observation was based on the ability of the researcher to be able to manipulate the organs or parts of the body with ease, and the organs being able to return to their normal form after manipulation. Morphological changes were also noted based on the pre-plastination features of the specimen. The shrinkage of specimens was based on a reduction in their weight after plastination.

3. Results and Discussion

A comparative dehydration study on piglet and kid cadavers as platinates through a room temperature and passive process was conducted. Preliminary results of the study show that the weight of kids as specimens after plastination shows that there is a 29.21% decrease in the weight of the kid dehydrated with acetone at room temperature and a 35.19% decrease in the weight of the kid dehydrated with increasing concentration of ethyl alcohol post-plastination. Similarly, there is a marked decrease of 23.81% and a 33.85% in the weight of piglets subjected to the room temperature acetone and ethyl alcohol post-plastination, as shown in Table 1. Note that ethyl alcohol dehydration significantly reduced weight compared to acetone. Similar results were also observed by Brown *et al.* (2002), where the average shrinkage of room temperature acetone-dehydrated specimens was 20.2%, while for room temperature methanol-dehydrated specimens, it was 22.6% [21]. Furthermore, specimens in Treatment were 5.98% more dehydrated (kid) and 10.04% more dehydrated (piglet) when compared to specimens in Treatment 1. This can be attributed to the fact that neonates have more body water, and since the piglets were approximately three months of age, their significant growth also reduced their water composition to 90%. According to Cherian (2019), water makes up one-half to two-thirds of the body mass of adult animals and more than 90% of the body mass of newborn animals [22].

Table 1. Pre- and post- plastination weight (g) of kids and piglets subjected to two different dehydration solutions.

Treatment	Pre-Plastination Weight	Post-Plastination Weight	Percent Difference
Kids Cadaver			
Treatment 1	825	584	29.21%
Treatment 2	1089	753	35.19%
Piglets Cadaver			
Treatment 1	3578	2726	23.81%
Treatment 2	2839	1878	33.85%

Note: Treatment 1: acetone dehydration; Treatment 2: ascending ethyl alcohol dehydration

In both treatment groups, plastination did not change the specimens' color. For the color changes, the skin of the kid specimen in Treatment 1 had a light brown color post-dehydration, then became slightly darker post-impregnation; however, the color of the skin became lighter post-curing (Fig. 1, A1-3), while no significant change in color was noted in specimens in Treatment 2 (Fig. 1, B1-3). The specimens in both treatment groups were flexible, and signs of shrinkage were evident as shown by the grooves of the thoracic cage post-curing (Fig. 1 A3, B3), but the morphological conformation of the animal was not compromised and remained intact (Fig. 1 A4, B4). The eyes had obviously shrunk; this was corrected by the researcher by infiltration of the eyeball with silicon sealant to fill in the space where the aqueous and vitreous humor are originally located to return the shape of the eyeball using a syringe.

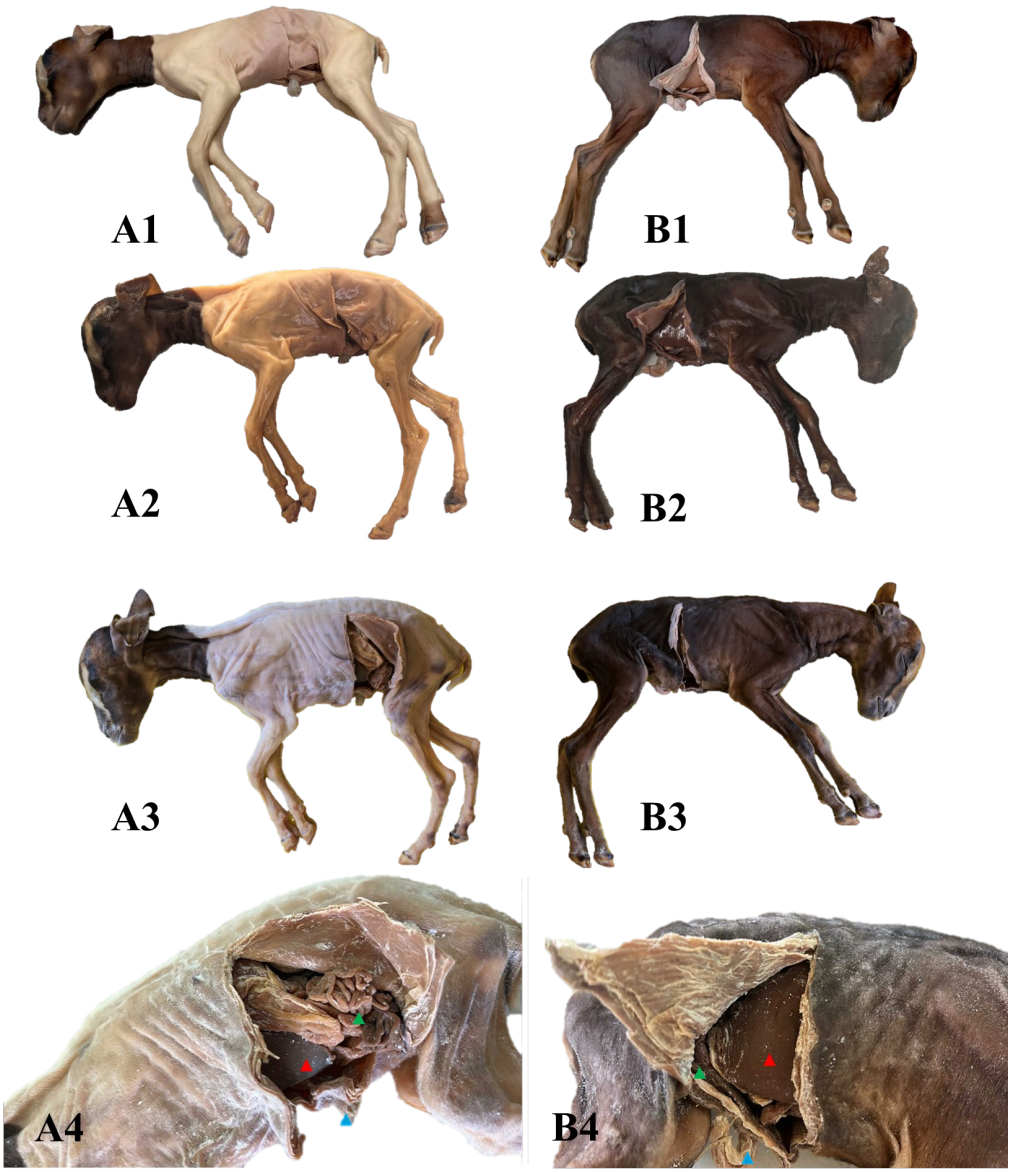

Figure 1. Photographs of kid plastinates showing the differences in color between treatment groups (A1-4: Treatment 1, B1-4: Treatment 2) post-dehydration (A1, B1), post-impregnation (A2, B2), and post-curing (A3, B3). Enlarged photograph of the visceral organs (A4, B4). In arrowhead: liver (red), jejunum (green), and umbilical cord (blue).

During the post-dehydration, there were no significant color changes noted in both specimens (Fig. 2 A1, B1). The muscles and visceral organs appear light to dark brown in color, while the adipose tissues appear white. Yellowish discoloration can be seen on the body of the piglet in Treatment 1 after it was subjected to impregnation. Furthermore, the color of the visceral organs varies from dark brown, like the spleen, to light brown in muscles and lungs. The visceral organs and muscles of the specimen in Treatment 2 exhibit a deeper brown color compared to those in Treatment 1. The lungs of the piglet in Treatment 2 appear darker when compared to the lungs of the piglet in Treatment 1 (Fig. 2 A2, B2; Fig. 3B). However, the color of organs in both treatment groups lightens post-curing with cornstarch (Fig. 2 A3, B3; Fig. 3B). It can be noted that the liver and diaphragm appeared dark brown after the impregnation process and changed to a lighter brown color in both treatment groups post-curing. The organs were firm to the touch and had no unpleasant odor from the plastinated specimens, even after 4 months post-plastination. This result is similar to that of Srisuwatanasagul *et al.* (2010), where dehydration by acetone gives a more natural color with no shrinkage of the heart specimens, while dehydration by ethyl alcohol gives a darker color and more shrinkage [13].

Shrinkage starts during the dehydration process; hence, it is an important consideration during plastination studies. Although a marked shrinkage, as shown by the marked reduction of weight of specimens post-plastination, was observed, the morphological features of the specimens showed no signs of degradation, and

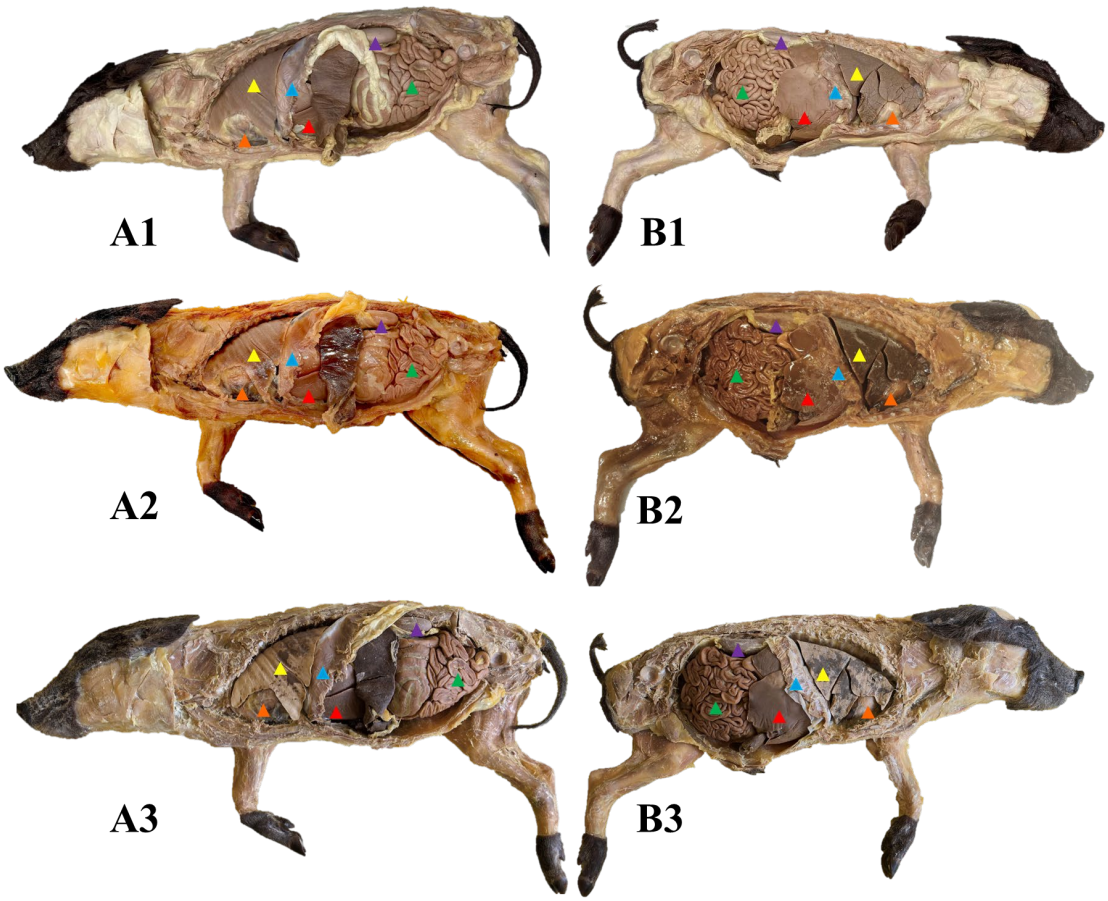

Figure 2. Photographs of piglet plastinates showing the comparison of specimen color between treatment groups (A-Treatment 1, B-Treatment 2) post-dehydration, post-impregnation, and post-curing with corn starch. In arrowhead: lungs (yellow), diaphragm (blue), liver (red), jejunum (green), kidney (purple), and heart (orange).

organs remained discernible, were dry, and had no odor even after 4 months of post-plastination. Similar results were observed by various studies utilizing locally available resources and the room plastination technique, where organ plastinates are odorless, completely biosafe, robust, dry, flexible, durable, natural in color and texture, can be used for a long time without decay, are lightweight specimens that are easy to carry, can easily be used for teaching, and demand significantly less maintenance than conventional cadavers. [10-19,26]

Currently, most anatomical specimens are preserved and stored in formalin solution. Although these formalin-immersed specimens are cost-efficient and can be maintained for long-term storage, they are typically stored in bulk containers or jars, which are difficult to handle in practical anatomy teaching courses [24] and carry a higher risk of exposure to formalin fumes, which can cause conjunctival and respiratory irritation. [23] Formalin-immersed specimens limit students to a hands-on learning experience, a valuable attribute of using plastinates as a learning resource. [20,23] Students' and professors' exposure to health hazards is decreased by the specimens' durability, odorlessness, and being free from dangerous substances. [16,25]

Since its introduction, this technique has gained considerable recognition in both medical and veterinary institutions for offering highly realistic representations of anatomical structures. [25,27] Thus far, Plastination has proven to be an almost ideal technique for long-term preservation of biological tissues, which can serve as specimens for practicing surgical techniques. [28] Plastination can provide a supplementary method to demonstrate anatomical differences and is an ideal method for long-term preservation of the most valuable preparations. In addition, plastinates are essential to complement the traditional dissection courses and contribute to a better preparation of postgraduates and clinicians [24].

5. Conclusions

In conclusion, this study demonstrates the effectiveness of room temperature plastination, comparing acetone and ethyl alcohol as dehydration solutions for preserving piglet and kid specimens. Both acetone and ethyl alcohol dehydration methods resulted in remarkable weight reduction, but the morphological features of the specimens were well-preserved. The plastinated specimens exhibited flexibility, firmness, no odor, and a natural appearance, with some color variations observed between the two dehydration methods. Moreover, ethyl alcohol can also be used as an alternative dehydration solution in place of acetone. Especially, acetone is classified as a controlled chemical, which makes it difficult to purchase. The results suggest that this alternative plastination method can be a valuable method for long-term preservation of anatomical specimens, could provide a hands-on learning experience for students, and reduce exposure to formalin. The preserved specimens can serve as potentially useful models for comparative anatomy studies, supplementing traditional dissection methods. Overall, this study highlights the potential of plastination as a reliable and effective technique for preserving biological specimens.

Author Contributions

Conceptualization, J.D.C.; Methodology, J.D.C.; Investigation, J.D.C.; Writing – Original Draft, J.D.C.; Writing – Review & Editing, J.D.C.; Funding Acquisition, J.D.C.; Resources, J.D.C.; Supervision, J.D.C.

Ethics Approval and Consent to Participate

Not applicable

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Conflict of Interest

The author declares no conflict of interest.

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