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Short Communication

FOETAL EMBALMING IN BUFFALO- AN UMBILICAL APPROACH

P.V.S.Kishore*, M.P.S.Tomar, K.Archana, N.K.B.Raju, D.V.V.N. Durga Prasad

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ABSTRACT: An umbilical approach was adopted to embalm and preserve an aborted buffalo fetus. The principle behind the present approach was to follow the natural course of fetal circulation. 10 % neutral buffered formalin was injected into the umbilical vein using a syringe. The umbilical arteries were ligated when the blood and interstitial fluid started to drain out. An incision was then made on the dorsal pedal artery as an exit for circulation. The fetus was tilted upside down to enable the distribution of formalin throughout the body by reaching the extremities due to gravitation. After ensuring complete perfusion of formalin, the umbilical vein and the dorsal pedal artery were ligated and the fetus was kept at room temperature for three days. The left body wall was reflected dorsally after a mid-ventral incision to expose the internal organs. All the organs were normal in color and consistency without any foul odor. The specimen was later stored in a cooler at 4°C, to avoid prolonged storage in formalin. Routine histological examination was also performed to ascertain the effect of fixation in different body organs which revealed normal histoarchitecture. The present approach of embalming ensured adequate fixation of the fetus.

Key words: Embalming, Perfusion, Buffalo fetus.

In Veterinary education, anatomists use cadavers to teach students, either by demonstrating prosected specimens or by dissections. One of the most important prerequisites for the use of cadavers is their appropriate preservation by embalming to maintain, as far as possible, a life-like state, and in the process, retaining the anatomic relationships required for dissection purposes (Dixit *et al*. 2005). In routine practices of veterinary anatomy, the healthy animal is sacrificed and embalmed which attracts the concerns of various animal welfare agencies (Kishore *et al*. 2019). In addition, the exposure to formalin preserved specimen causes irritation to the mucous membranes of the pharynx, upper respiratory tract and eyes resulting in discomfort to handlers (Dixit *et al*. 2005, Banoo *et al*. 2016). To avoid these ethical and health issues, some authors proposed alternative artificial models which compromise the realistic experiences by observers (Pederson 2002, eSilva *et al*. 2008, Kishore 2018). However, skill laboratories were proposed to be developed by the institutions to train students on interactive alternative models (Jukes and Chiuia 2003). Further, an ethical sourcing of animal cadavers for education and training was also defined in the interNICHE policy which may fulfill all the challenges faced with alternate teaching practices without raising ethical concerns (Jukes and Martinson 2008).

In this connection, the Willed Body Program was established to minimize sacrifice of animals for dissection and to prepare humane alternatives in veterinary education. In continuation to our previous works (Kishore 2018, Kishore *et al*. 2019), an aborted buffalo fetus was embalmed for teaching veterinary embryology.

The fixation and preservation of embryological specimens is of great importance. Traditionally, these specimens are immersed in 10% formalin after cavity embalming and spot injection for display in museum jars. But this method does not ensure adequate fixation of bigger fetuses of large animals. The fetuses fixed and stored in the traditional way are not found suitable for developmental studies which is a matter of great concern. Therefore, the present study was carried out to ensure better fixation and preservation.

Department of Veterinary Anatomy, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh -521 102, India.
**Corresponding author. e-mail: pvskishore_1963@yahoo.com*

Procedure

A five months old aborted male buffalo fetus (which is normally discarded and destined for disposal) was procured immediately from the livestock farm complex of NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India. The crown vertebral rump length (CVRL=34.5 cm) was measured to ascertain its age (151 days) by using the Soliman's formula (Soliman 1975) as follows:

Y= 28.66 + 4.496 X (CVRL< 20 cm)

Y= 73.544 + 2.256 X (CVRL > 20 cm)

Y is age in days and X is CVRL in centimeters.

The fetus was initially stored in a freezer overnight. After thawing, it was washed with tap water to remove the blood stains and debris. Perfusion with 10% neutral buffered formalin was done through the umbilical vein using a 50 ml. syringe. The umbilical arteries were ligated when the fluid started to drain out of them and then an incision was made on the dorsal pedal artery as an exit for circulation and to ensure that formalin reached the distal parts of the limbs. The body was tilted upside down and vice-versa to enable proper distribution of formalin throughout the body as the circulatory system was non-functional. As the embalming fluid circulated, the skin became pale and the fetus appeared bloated with stiffened

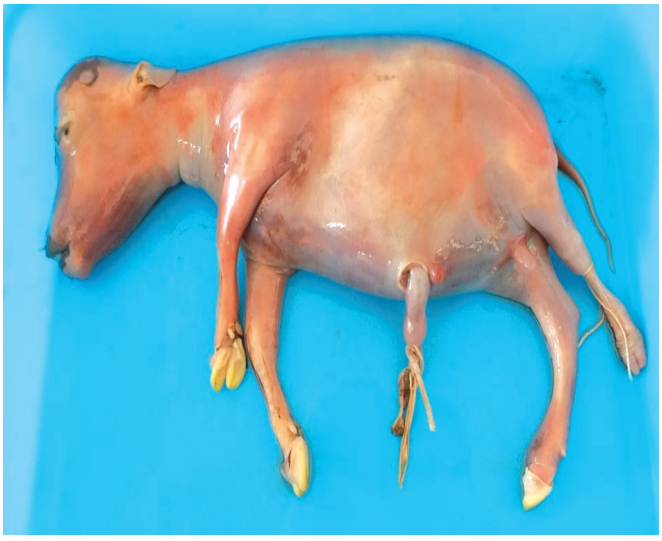

Fig. 1. Embalmed fetus after umbilical perfusion.

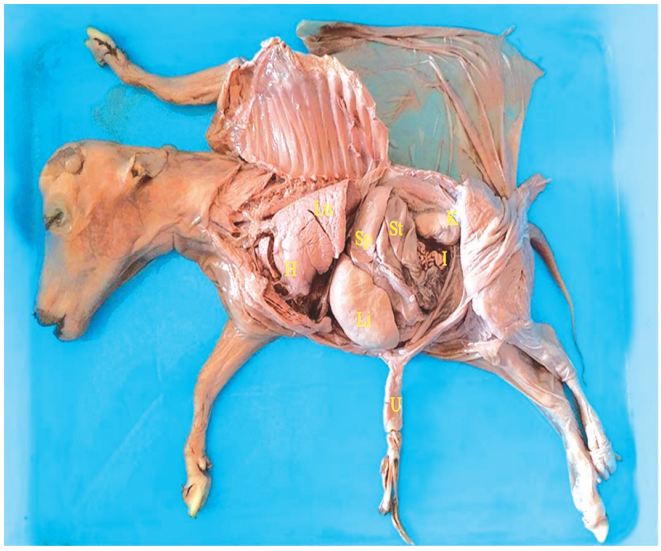

Fig. 2. Dissected fetus showing *in situ* organs.

[H=Heart, Lu=Lung, Sp=Spleen, Li=Liver, U=Umbilical cord, K=Kidney, I=Intestines, St=Stomach].

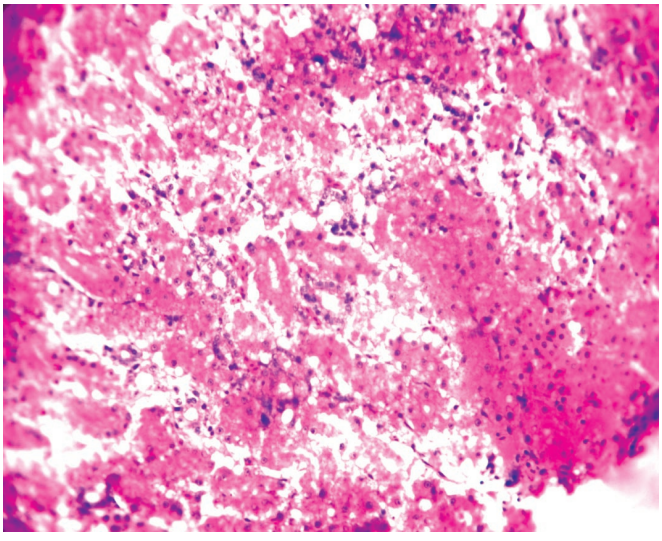

Fig. 3. Photomicrograph of developing liver showing light and dark hepatocytes.

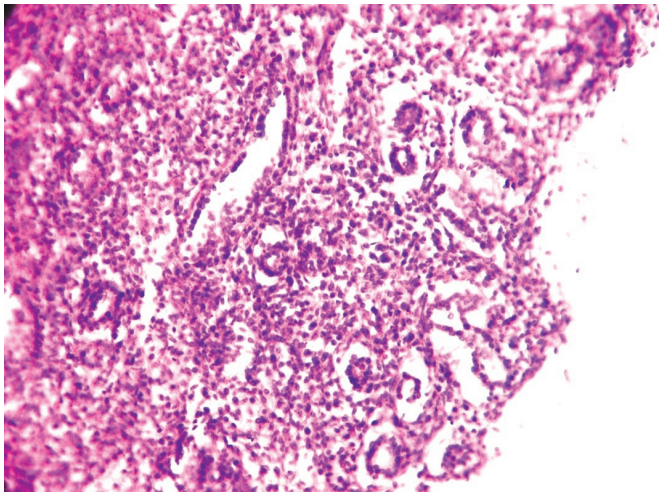

Fig. 4. Photomicrograph of developing lungs showing canalicular stage.

limbs, tail and erect ears (Fig. 1). After complete perfusion, the dorsal pedal artery and the umbilical vein were ligated. The fetus was kept at room temperature for three days. During this period, the perfused fetus was covered with a moist cloth and was moistened periodically. The left body wall was reflected dorsally after a mid-ventral incision to observe the internal organs for their color, gross appearance, consistency and odor. To ascertain the effect of fixation, tissue pieces from different body organs *viz*. lungs, liver, spleen and kidney were collected and processed for routine histological examination. The specimen was then stored in a cooler at 4°C to avoid prolonged storage in formalin and was observed periodically.

Discussion

Generally, embalming involves the injection of embalming fluid into either the common carotid artery or the femoral artery and blood is removed from the jugular vein (Ajileye *et al*. 2018). However, in normal fetal circulation blood passes into the fetus through the umbilical vein which finally enters the posterior vena cava and later reaches to heart. The circulated blood travels back to the placenta through the umbilical arteries for oxygenation (Carlson 1985, Konig and Leibich 2004). To mimic the normal course of circulation, formalin was injected through the umbilical vein in the present investigation, which ensured the proper distribution of fixative in all the body parts including the internal organs. The perfused specimens were subsequently kept at room temperature for three days to attain complete fixation. The fetus was dissected and no abnormality was observed in the gross appearance, color, consistency and odor. However, shriveling of the skin was encountered which may be due to the lower water retention ability of fetal skin resulting in loss of moisture (Fig. 2). The organs retained a life-like condition to provide hands-on-experience to the students for laboratory teaching. The fetus was then preserved in a cooler at 4°C to avoid immersion in formalin for long term storage. Even after a month's storage in cooler at 4°C the gross characteristics appeared normal as perfusion of the fetus through the umbilical vein ensured proper fixation.

Routine histological examination revealed normal histoarchitecture in the liver, kidney, lungs and spleen of the fetus. In liver, the central vein, portal triad and radiating hepatic cords were observed. The hepatic cords showed alternate light and dark cell pattern of hepatocytes. An extensive sinusoidal hematopoiesis was also noticed (Fig. 3). These observations were accordance with the findings of Sahoo *et al.* (2017).

The lungs showed partially distended airways with more compact arrangement of the parenchyma showing canalicular stage of its development. Branching of tubules was observed and the lining epithelium of air spaces showed transition between columnar to cuboidal type (Fig. 4). In spleen, lymphocytic condensations were observed around arterioles. Splenic sinuses were in the beginning stage. All these histological observations were in accordance with the findings of Ernst *et al.* (2011) in human beings. In kidneys, well-marked lobulation with nephrogenic zone at the periphery of a thin cortex was observed with S shaped body of nephrons as described by Sarma and Ahmed (2007) in crossbred pig and Fayez *et al.* (2014) in rabbit.

These observations confirmed that fetal embalming by perfusion through the umbilical vein fixed the tissues effectively. The specimen prepared in this manner can therefore be used for teaching veterinary embryology. Embryological details can well be appreciated in this specimen with a great potential for enhancing embryology teaching compared to the digital and resin alternatives. This technique is cost-effective; it satisfies the ethical and health concerns as a dead fetus destined for disposal was preserved successfully. This method also minimizes the repeated washing of specimens taken out from the formalin tank for use in the laboratory. These specimens can further be used for plastination techniques (Elnady 2016).

In this perfusion technique, except for 10% neutral buffered formalin no other chemicals were used. This is advantageous compared to the expensive technique of preparation of glycerinated and plastinated bodies (eSilva *et al*. 2008) and the Elnady technique which also preserves tissues but as dry specimens with the use of chemicals (Elnady 2016).

The embalmed fetus was realistic, soft and flexible and had no offensive odor. Its internal organs retained a life-like condition to provide *in situ* appearance to the students. They served as valuable specimens for teaching veterinary embryology. This technique can be used for preparation of anatomy specimens in future from the organs discarded after slaughter or from naturally dead donated or disposed cadavers. The storage of the embalmed fetus in a cooler at 4 °C for periodical use is also an alternative to the long-term storage in hazardous formalin tanks. The specimen can further be used for preparation of dried specimens by plastination techniques.

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