

SHORT COMMUNICATION

Gross anatomical and radiographic findings of museum preserved buffalo calves conjoined twin for learning purposes

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ABSTRACT

Objective: Current study aimed for documenting a rare case in buffalo calves in Egypt about embryogenesis anomalies followed by dystocia.

Material and methods: The stillborn calf was preserved using Elnady technique. The twin was radio-graphed in ventrodorsal position and several digital images were taken due to large size of the twin and were stitched together using Adobe Photoshop. The preserved twin specimen was kept in a wooden glass cabinet at the Anatomy Museum, Faculty of Veterinary Medicine, Cairo University with a booklet described the case and a video CD for dystocia operation steps in cows and buffalos.

Results: The external features of the twin were classified as dicephalus, tetrabrachius, Parapagus, and bipus. Radiographic study showed that the twin had two vertebral columns that converged at the lumbosacral region to come adjacent to each other with absence of sacrum and coccygeal vertebrae. The trunk cavities showed two sets of heart and lung, two stomachs; left one in the abdominal cavity and the right one in thoracic cavity. The two duodenum fused together to form one set of intestine terminated in persistent cloaca with the two ureters originated from the single set of two kidneys. It had one fused liver received the two umbilical veins and had two gall bladders.

Conclusion: The present study recommended the preservation of rare specimens using Elnady technique for long term to facilitate student's interaction with one of the rarest cases in buffalo that causes dystocia.

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Introduction

Buffaloes were usually uniparous with one calf per pregnancy. Twinning rate in buffaloes ranged from 0.0002% up to 0.01% in most of world buffalo breeds [1,2]. The conjoined twin incidence in bovine is about 0.039% with a suggestion of one conjoined twin in each 100,000 births [3]. Different types of conjoined twin were reported in buffalo [4–18] as rare separate cases.

Fetal monsters including conjoined twins were represented 7.9%–12.8% causes of dystocia in river buffalo. The interference with such cases was either through fetotomy or caesarian section. Caesarian section had 45% lower survival rate than fetotomy. Survived cases had 36% conception rate in dams with caesarian

section deliveries versus 23% in dams with fetotomy deliveries [19].

Conjoined twins described as two identical fetuses in twin pregnancy were linked with parts of the body and shared some organs ranging from a single set of viscera to almost all of internal organs. Most conjoined twins' cases were either stillbirths or miscarriage [20].

Due to unavailable experimental models, the exact embryogenesis of conjoined twins is still unknown. There were two theories named fission and fusion. Fission theory described splitting of single embryonic disc in the same manner as in twinning but in later time than in the normal twinning cases causing incomplete separation of the twins [21]. The fusion theory was more likely as it explained

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different types of conjoined twins. The fusion theory suggested single yolk sac with two separate embryonic discs from monovular pregnancy and fusion happened at sites disrupted from ectoderm as fusion at level of septum transversus resulted in thoracopagus and omphalopagus twins, the fusion at the level of oropharyngeal membrane resulted in cephalopagus twins, and the fusion at the cloacal membrane resulted in ischiopagus and parapagus twins [22–24].

The present study aimed for documenting a rare case in buffalo calves in Egypt and to preserve the twin calves as a museum specimen to educate students and visitors of the anatomy museum about one of embryogenesis anomalies which is one of the main causes of dystocia in ruminants.

Materials and Methods

Ethical approval

A stillbirth conjoined buffalo calves twin was delivered using cesarean section at the Theriogenology & Gynecology clinic of the Fac Vet Med, Cairo University, Egypt. The twin was stillborn and was presented as an ethical source for research.

Specimen preservation

The twin was preserved using Elnady technique [25] which is a modified plastination technique registered for patency in Egypt (#2051/2014), and the process steps followed were all at room temperature. The twin was fixed by cannulation through umbilical vessels and injected with 20% formalin solution and left for 3 weeks then rest of steps: dissection, dehydration, impregnation, and curing were followed according to [25].

Radiography

The Twin was radio-graphed in ventrodorsal position using Poskom digital X-ray unit model No. PXP-40HF (Goyang, Korea) at exposure factors 45 kv and 13 mAs. Several digital images were taken due to large size of the twin and were stitched together using Adobe Photoshop CC.

Keeping as museum specimen

The specimen is preserved at room temperature in labeled display wooden glass cabinet (130 cm long, 110 cm width, and 60 cm height) at the Anatomy museum, Fac Vet Med, Cairo University, Egypt.

Results

External anatomy

The twins were classified as dicephalus (had two heads and necks), tetrabrachius (had four full forelimbs), Parapagus

(the twin merged in one common thoracic cavity, one abdominal cavity, one pelvic cavity), and bipus (The twin had only one pair of pelvic limbs) (Fig. 1A and B). Two vertebral columns extended along the twin entire length with one tail divided into two ends (Fig. 1D). The twin had one closed anus. Also, the twin had from the ventral view single set of prepuce and scrotum contained two small testes palpated at the neck of scrotum (Fig. 1C).

The single umbilicus contained two normal umbilical arteries connected to persistent cloaca and two umbilical veins that entered the fused liver (Fig. 1C).

Trunk cavity

The trunk cavity was divided into thoracic and abdominal cavities.

The thoracic cavity contained two tracheas leading to two sets of lungs (Fig. 2A). The cavities contained two hearts each enclosed in separate pericardium (Fig. 2A). The right twin esophagus entered right stomach which

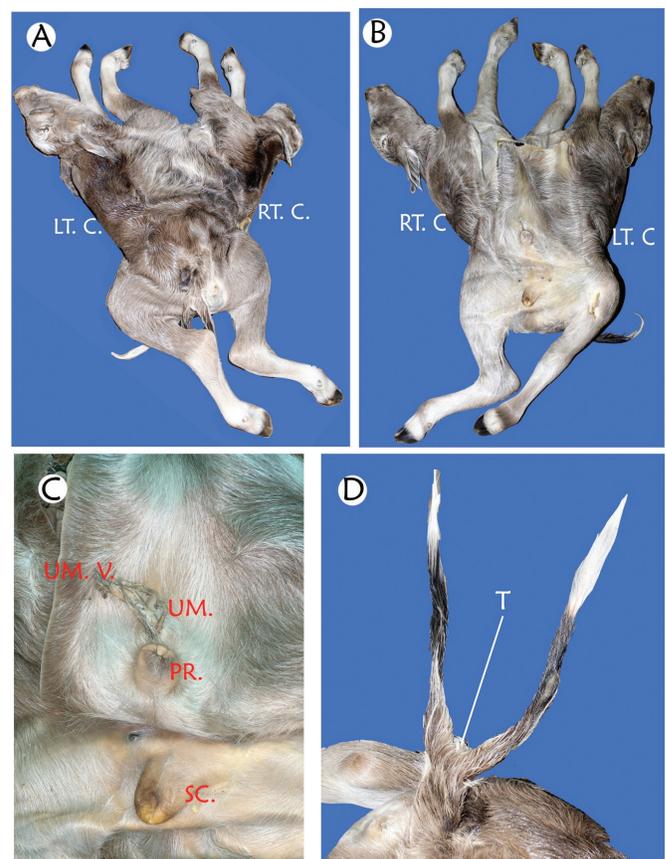


Figure 1. External features of Plastinated male buffalo conjoined twin. (A) Dorsal View of entire twin. (B) Ventral view of the entire twin. (C) Ventral view of the perineum region. (D) Dorsal view of bifid tail. LT.C: Left calf. RT.C: Right calf. Um. V.: Umbilical vessels. UM.: Umbilicus. PR.: Prepuce. SC. Scrotum.

was fully herniated inside the thoracic cavity with the right spleen attached to its dorsal surface (Fig. 2A). The right duodenum departed the stomach and penetrated through diaphragm to pass dorsal to liver into abdominal cavity (Fig. 2B). The left twin esophagus passed through diaphragm to the abdominal cavity.

The abdominal cavity contained fused two livers with two gall bladders just caudal to the diaphragm (Fig. 2A and B). Caudal to the liver on the left side, the left twin stomach with the left spleen attached to its dorsal surface and from the abomasum, the left duodenum departed and extended for almost 8 cm (Fig. 2B). The right and left duodenum were fused and continued as one set of jejunum, ileum, and large intestine (Fig. 2B).

The caudal end of the large intestine united with the urinary bladder that terminated into a large pouch at the pelvic inlet “persistent cloaca” while rectum was absent (Fig. 2C and D). That arrangement exposed the incomplete development of the hind gut as well as the urinary system and tracts. The twin had a single urinary system with two kidneys and the ureter terminated in the persistent cloaca (Fig. 2D).

Osteology

The bone study depended entirely on the radiographic imaging to preserve the cadaver as an anatomy museum specimen. The twin had fully developed two sets of skulls and mandible, four full forelimbs but the fractures in two of

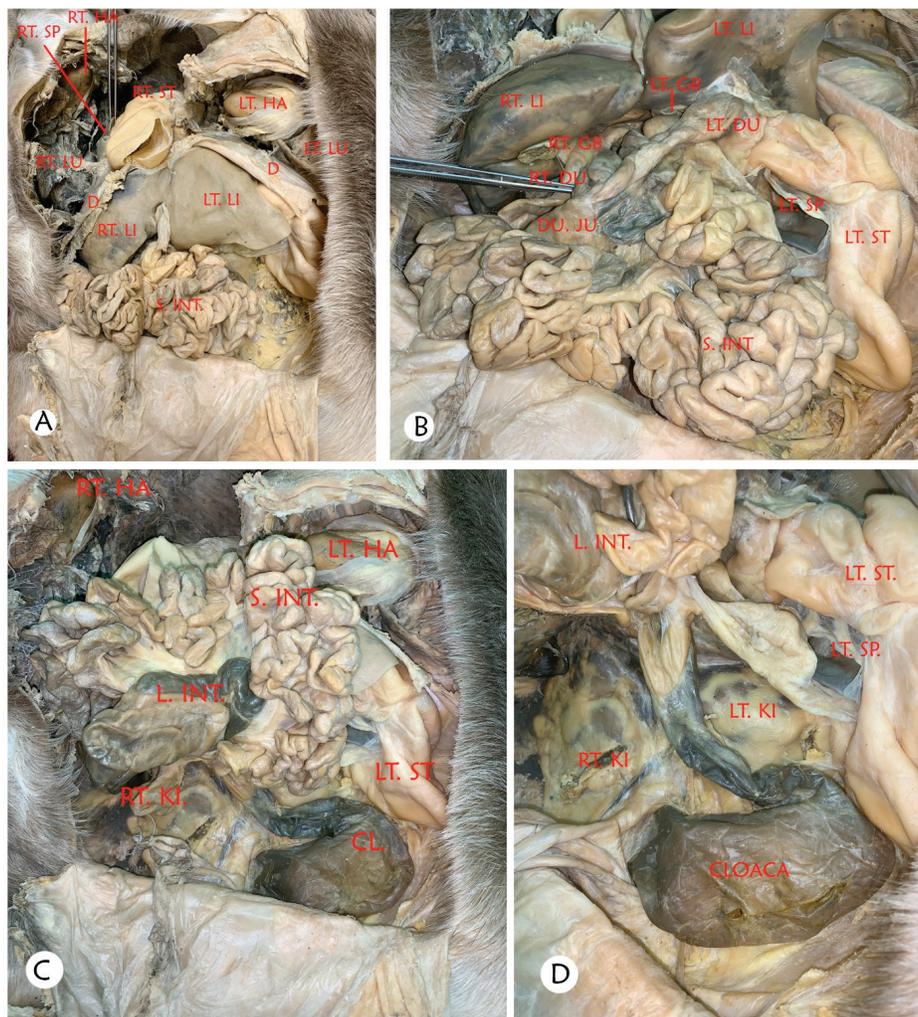


Figure 2. Internal features of Plastinated male buffalo conjoined twin. (A) Ventral view of entire twin Trunk cavity. (B) Ventral view abdominal cavity showing the liver and the two duodenum. (C) Ventral view of the abdominal cavity with reflected intestine. (D) Ventral view of the caudal part of the abdominal cavity. RT.HA.: Right Heart. LT.HA: Left heart. RT.LU.: Right lung. LT.LU. Left Lung. RT.ST.: Right Stomach. RT.SP.: Right Spleen. D. Diaphragm. RT.LI: Right liver. LT.LI: Left liver. S.INT.: Small intestine. RT.GB: Right gall bladder. LT.GB: left gall bladder. R\LT.ST.: Left Stomach. LT.SP.: Left Spleen. RT.DU: Right duodenum. LT.DU: Left duodenum. DU.JU: Duodenojejunal junction. L.INT.: Large intestine. RT.KI: Right kidney. LT.KI: Left kidney. CL.: Cloaca.

the forelimbs was due to excessive bulling during cesarean section and two sets of ribs and two sternums (Fig. 3A). The twin had two vertebral columns that converged at the lumbosacral region to come adjacent to each other with absent sacrum and absent coccygeal vertebrae (Fig. 3B). The twin had one pelvis with two fully developed hind limbs.

Learning module

The twin specimen after preserved by the Elnady technique was kept in a wooden glass cabinet (Fig. 4A). The cabinet included the specimen; a booklet described the case with photographs and review on the possible causes of dystocia in buffalos and cows as well as a CD played a video for dystocia operation steps in cows and buffalos either through caesarian section or fetotomy. Those learning tools used by under and postgraduate students in an integrated manner for better understanding, how to study and manipulate with dystocia problem in buffalos and cows (Fig. 4B).

Discussion

The conjoined twins in buffalo were previously documents by scientists and its names varied according to the fusion place. The conjoined twin in the present study was the first reported case in Egypt and was classified into Dicephalus Tetrabrachius, Parapagus Dipus according to classification provided by Spitz et al. [24]. The common

cases of conjoined twins were thoracopagus and thora-co-omphalopagus, while the lowest were the parapagus cases [26]. The radiographic proof of absence of sacrum and coccygeal vertebrae in the present study were not reported in similar cases in buffalo [13] and in cow [3]. The present study twin was associated with retained cloaca which presence was explained as a failure for migration of the mesodermal cells of cloacal septum, which prevented cloacal division into a dorsal anorectum and a ventral urogenital sinus as well as loss in caudal cell mass [27]. The caudal vertebral column formed by secondary neuralization of the caudal cell mass just before the division of cloaca by cloacal septum. Thus, cell loss in the caudal cell mass could explain the association of absence of caudal part of vertebral column and the presence of retained cloaca in the present study [28,29].

Through the present study, the twin had two esophagus, two stomachs with the right one was herniated in the thoracic cavity and two duodenum, the duodenum of both sides fused together in a single jejunum, ileum, and large intestine terminated in cloaca which similar finding in Parapagus Holstein calf [3] and monster buffalo calf [8], but these cases had rectum and both stomach were present in the abdominal cavity behind diaphragm. Other parapagus twins [4,17] had one set of alimentary tract and a thoracopagus with one set of alimentary tract with two rectum [13], while thoraco-sternopagus had two full sets of alimentary tract [6,14].



Figure 3. Radiographs of male buffalo conjoined twin at exposure factors 50 kv and 13 mAs. (A) Formed (Stitched) image of the entire twin in ventrodorsal position. (B) Dorsoventral view of the pelvic region of the conjoined twin showing termination of two vertebral columns by the lumbar vertebrae and absence of sacrum and coccygeal vertebrae. CE: Cervical vertebrae. TH: Thoracic vertebrae. LU: Lumbar vertebrae. IL: Ileum.



Figure 4. Learning module for the plastinated conjoined twin. (A) The conjoined twin specimen kept in a wooden glass cabinet. The cabinet included the specimen, with the booklet and the CD. (B) The learning tools while used by under and postgraduate students.

The present study twin had a fused liver with two gall bladder was similar in other trunk conjoined twins as parapagus Holstein calf [3], thoraco-sternopagus buffalo calf [14], and thoracopagus buffalo calf [10,16], while conjoined twins had two separate livers [9].

The present case had one pair of kidneys as similar to Vanderzon et al. [3] and Dutt et al. [15], while some other reported two sets of kidneys [13].

In agreement with Tolba [30], who emphasized that, the anatomy museums were not only the place to keep valuable stuffs but also it was a place of self-learning through an interactive libraries and learning carrels. One of the objectives of the current study was to use the anatomy museum, Faculty of Veterinary Medicine, Cairo University as a place of learning the most probable causes of dystocia though identified one educational problem of the dystocia reasons which was “twinning” by bringing multiple educational materials to facilitate self-learning.

Due to the objective of long-term preservation of the twin as a museum specimen, the detailed osteological and vascular studies were limited which was described in Holstein calf [3].

Conclusion

The current twin anatomical features explained the impossibility of natural parturition and subsequently dystocia.

The long-term preservation using Elnady technique of such rare specimens is recommended for continuous veterinarian’s education about such anomaly and why fetotomy and caesarian sections are suggested.

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Conflict of interests

The authors declare that they have no conflict of interest.

Authors’ contribution

AT planned the study. AT and SD drafted the manuscript. HF interpreted the data and took a part in preparing the manuscript. SD visualization, reviewing, and critical checking of this manuscript.

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