Dicephalic Parapagus Conjoined Twin Fleckvieh Calves

Kyle C. Johnson, MS, MPH
Mississippi State University
College of Veterinary Medicine
Class of 2019

Clinicopathological Conference
October 26, 2018

CPC Advisor: Heath King, DVM, DACT

Introduction

Congenital abnormalities are those derangements that occur during the gestational period and are apparent at birth[24]. These defects have been documented in humans, domestic animal species, and wildlife and are usually a result of genetic or environmental factors or both[5, 18]. The exact etiology of these congenital abnormalities is often unknown. Regardless of the cause, these abnormalities are often incompatible with life, resulting in the death of the neonate. An abnormally developed fetus can also pose a danger to the dam due to an increased risk of dystocia and subsequent maternal morbidity and mortality[14, 17]. Furthermore, congenital abnormalities can be a source of production and economic loss for livestock producers. Given their possible negative impacts on neonatal morbidity and mortality, as well as, their effects on producers, it is prudent that veterinarians be able to recognize congenital abnormalities and understand their epidemiology and pathophysiology to possibly minimize future losses.

Of the domestic species, the incidence of congenital duplication is highest in cattle with an incidence of one anomaly in 100,000 births[7, 18]. Twinning, a prerequisite for duplication, has an incidence of 1-to-7% in cattle, although this figure has great variability in the literature. Twinning is more common in high producing dairy cattle compared to beef cattle[22]. The etiologies of twinning in cattle are well understood, but generally go undiagnosed in individual animals in the production setting. In humans, a link has been made between the use of exogenous hormones and the development of conjoined twins. This could prove to be of high importance to the cattle industry due to the increasing use of assisted reproductive technologies (ARTs) and their use of exogenous hormones for synchronization, superovulation, among others[20]. The case presented here involves congenital duplication in a cow that was part of a producer's embryo transfer (ET) program. The following discusses the patient's history, presentation and case summary. Furthermore, the necropsy findings and relevant pathophysiology are also discussed.

History and Presentation

Roxanne is a six-year-old, German Fleckvieh cow that presented on emergency to Mississippi State University College of Veterinary Medicine's (MSU-CVM) theriogenology service on February 27, 2018 for dystocia. Roxanne was a multiparous animal of superior genetic quality and was utilized in the operation's ET program as a donor cow. The patient had calved previously without incident. There was no report of twinning or of any congenital abnormalities in the calves born previously. No other animals in the herd were showing similar signs. No other pertinent medical history was available.

On presentation, the patient had been in labor for over an hour. A veterinarian had vaginally palpated the patient and attempted to manipulate the calf into a presentation suitable for an assisted delivery. A powder based obstetric lubricant (J-lube) was used to facilitate this attempt to manipulate the presentation of the calf. Additionally, two hind limbs that were exteriorized from the birth canal had been removed in the field by the referring veterinarian. The patient was unloaded from the trailer without incident and voluntarily ambulated into the hospital. The patient was placed in a squeeze chute and was restrained for evaluation and treatment. The patient's vital parameters were within normal limits (T=102.5 F, pulse = 60 beats per minute, respiration = 32 breaths per minute). Her mucous membranes were pink and moist, and her capillary refill time was less than 2 seconds. Due to the emergent nature of the patient's condition on presentation, a full physical exam was not performed, but no gross abnormalities were noted.

Two pelvic limbs were visible protruding from the vagina and the surrounding vaginal tissue was swollen and edematous. Vaginal palpation revealed that the fetus was oriented in a posterior-longitudinal, dorso-left-ilial position with bilateral extension of the pelvic limbs. The withdrawal reflex was absent in the hindlimb of the calf suggesting that the calf was not viable. Furthermore, following vaginal palpation, it was determined that the calf could not be extracted, and that surgical intervention would be necessary. Despite a nonviable calf, it was determined that a Cesarean section would be performed, in lieu of a fetotomy, due to the size of the calf, the limited space in which to operate and the subsequent risk of peritoneal contamination. Given the age, parity, and condition of the dam and fetus, a recumbent ventral midline celiotomy was performed as opposed to the standard flank approach[19].

While adequately restrained, an area over the sacro-caudal region of the spine was clipped and aseptically prepped. A caudal epidural was performed using 2% Lidocaine. The patient was sedated with a combination of 0.03mg/kg butorphanol, ketamine0.1mg/kg and 0.05mg/kg xylazine which was administered intravenously. Once adequately sedated, the patient was placed in dorsal recumbency. The ventral midline was clipped and aseptically prepped. Then a line-block with 2% Lidocaine was performed prior to the initial incision.

A ventral-midline approach and caesarean section with uterine repair was performed. The calf was dead on delivery and was a fetal monster, the specifics of which will be discussed in detail below. Once the calf was removed, the uterus and abdomen were copiously lavaged with isotonic saline to which oxytetracycline powder and carboxymethylcellulose lubricant had been added. The carboxymethylcellulose was added to prevent adhesions and oxytetracycline was added prophylactically in the face of likely contamination of the abdomen. The uterus and several defects were closed using ChromicGut suture in a Utrecht pattern. The patient was given one dose of 60ml of Procaine Penicillin G, 300,000 IU/mL, intraabdominally prior to closure. The main uterine incision was oversewn using a horizontal mattress pattern. The body wall was closed using a simple interrupted cruciate pattern. The subcutaneous tissues and skin were closed with a continuous suture pattern.

The patient recovered from the procedure without incident and was given one dose of Procaine Penicillin G, 22,000 IU/kg, intramuscularly. The patient received 1.1 mg/kg of meloxicam orally, 1.1 mg/kg of flunixin meglumine intravenously, and 6.6 mg/kg of ceftiofur CFA subcutaneously as directed by the label. Lastly, the patient was given two doses of oxytocin intravenously (one following the procedure and one the next day). The patient was discharged three days later with two doses of meloxicam to be given as discussed previously.

Necropsy Findings

Following surgical delivery of the nonviable calf, the body was submitted to the MSU-CVM's diagnostic laboratory service for necropsy. The body was composed of two nearly complete calves. This type of presentation is referred to as *diplopagus*, meaning that the twins are conjoined and each have nearly complete bodies, although there may be some sharing of organs and other anatomical structures. The specimen possessed two complete heads, four complete thoracic limbs, and four complete pelvic limbs. The calves each had an independent vertebral column but shared a sternum and were thus joined at the thorax. As mentioned previously, one calf had its pelvic limbs removed in the field prior to presentation to the hospital. In accordance with the naming convention as outlined by Potter and Craig (1975) and Hiraga and Dennis (1993), this specimen is an example of dicephalic, thorocopic, tetraspusal, tetrabrachial twins. For the ease of identification and discussion, the complete calf (with all four limbs) will be referred to as Calf 1. The calf that had its pelvic limbs removed prior to necropsy will be referred to as Calf 2.

On presentation to the necropsy service, Calf 2's pelvic limbs were missing, as was discussed previously. In addition to the pelvic limbs, a substantial portion of the musculature covering the dorsal aspect of the vertebral column, as well as some of the abdominal wall was also absent. Due to the absence of part of the abdomen of Calf 2, the exact amount of connection between the two animal's abdomens was unable to be determined. Calf 2 had bilateral anopthalmia. The nares were incomplete ventrally and contributed to the complete cheilognathopalatoschisis. No gross abnormalities were noted on the face, head and neck of Calf 1. Each calf had a grossly normal esophagus, and trachea which was connected to an individual set of lungs. The lungs of both animals were uninflated, purple, and subjectively heavy. The brains were grossly normal.

In the thorax, there was a single heart that communicated with the lungs of both calves. The entire right heart (ventricle, atrium and auricle) were severely dilated, but no signs of hypertrophy were noted. An atrial and ventricular septal defect were present. The aorta communicated with the right ventricle and was overriding. On the external surface of the heart, pale, white, linear structures were observed throughout the myocardium.

In the abdomen of Calf 2, most of the organs were missing, which is likely attributable to the previous removal of the pelvic limbs. The only remaining organs in the abdomen were a single kidney and a section of the colon. The kidney was small and had decreased lobular distinction. The colon was grossly unremarkable. The abdominal contents of Calf 1 were grossly within normal limits. Severe scoliosis of the lumbar spine was noted in Calf 1. Suspected spina bifida with a myelomeningocele was observed in the caudal lumbar spine of Calf 1. Severe fetal anasarca was also noted during the necropsy.

Pathophysiology

Before discussing the potential etiologies of the congenital derangements observed in this case and their respective etiologies, it is prudent to discuss normal fertilization and subsequent development of an embryo. Following copulation and successful fertilization, the fusion of the male and female pronuclei from the sire and dam, respectively, results in the formation of the embryo. It is relevant to discuss some terminology here as some terms are often used interchangeably, but incorrectly. An embryo is an animal in the early stages of development, but that is not yet identifiable as a unique species[21].An embryo also describes an organism that has not yet completed organogenesis, a distinction that is reserved for a fetus which occurs at approximately 42-45 days following conception in cattle[15]. Furthermore, Senger (2003) defines a fetus as an animal at a particular stage of development when it can finally be recognized as a member of its of species. A conceptus refers to all embryologic tissues to include other tissues such as chorion and amnion[15, 21].

In the early stages of embryologic development, the embryo becomes the zygote and begins to undergo division known as cleavage. The first division results in two cells, which are known as blastomeres. The blastomeres undergo further iterations of division. Until a certain point, each of these blastomeres has the potential to develop into a unique individual which is known as totipotency. Once the blastomeres have had sufficient divisions to result in 16 cells, they are no longer considered totipotent[21].

Twin animals born in this manner, as a result of the totipotency of two blastomeres, are referred to as monozygotic twins or identical twins[8]. Such events occur in roughly 1% of beef cattle calvings and 4% of dairy cattle calvings[10]. In dairy cattle, the twinning rate has increased in the past several decades to approximately 9% and this increase has been linked to genetic selection and management for increased milk yield[1, 22]. Because of advances in nutritional and production management, a decrease in embryologic loss of twins has resulted in an increase in live twin births[1].

At approximately four days following conception, the bovine embryo has divided several times and has amassed 16 cells and moves into the dam's uterus from the oviduct[12]. At this point, the blastomere has become a morula. Tight junctions form between the cells on the perimeter, because of increased pressure between these outer cells and the zona pellucida. The cells toward the center of the morula are faced with less pressure and gap junctions. The purpose of tight and gap junctions is to facilitate cell-to-cell communication. The tight junctions also allow for fluid accumulation within the zona pellucida resulting in a blastocoele[21].

As fluid continues to accumulate within the blastocoele, an appreciable cavity begins to form. This results in the formation of the blastocyst. The formation of this cavity within the blastocyst leads to the development of two distinct cell populations. The first is the inner cell mass (ICM), which will ultimately give rise to the body of the embryo. The other is the trophoblast, which will give rise to the chorion, the fetal contribution to the placenta. The blastocyst eventually secretes proteolytic enzymes that degrade the zona pellucida. Once a fissure develops in the zona pellucida, the blastocyst can escape the zona pellucida and become free floating in the uterus prior to implantation into the endometrium[15, 21].

At this point in development, the embryo is a flattened disc known as the embryonic disc. At one poll of this disc lies the oropharyngeal membrane and opposite is the cloacal membrane. The notochord lies in the center of the disc and results in the formation of the vertebral column later in development[25, 26]. The embryonic disc undergoes folding in the longitudinal and sagittal planes to form the major body architecture around the three germ layers, the endoderm, mesoderm and ectoderm[25]. Once the two discs are formed in the event of monozygotic twins resulting from totipotent blastomeres, then congenital duplication can occur. The further differentiation and growth of these layers into subsequent tissues, structures and organs is immensely complex. It is only prudent to discuss development to this phase as this is where the congenital duplication occurs in monozygotic twins.

It is important to highlight one important premise to understand before discussing how congenital duplication occurs. This is that intact ectoderm will not fuse with intact ectoderm and thus there are certain anatomical sites at which the fusion can occur[25]. Next, there are two competing theories on how congenital duplication occurs, fusion and fission. The fission hypothesis asserts that there is incomplete separation of the two embryonic discs. The fusion theory proposes that the duplication occurs when the two originally separate embryonic discs fuse. As mentioned above, this can only occur in the absence of intact ectoderm[26, 27]. In diplopagus animals (conjoined twins), the congenital duplication is nearly always homologous, meaning like structures are fused, head-to-head, thorax-to-thorax, and so on[20, 26]. Interestingly, duplication in cattle is more often located in the cranial region of the body. The latter is true in sheep and goats, where caudal duplication is more common[20]. The duplication also has an affect on the fetal membranes and results in the sharing of these tissues by the neonates when they do not divide properly. The placentas seen in congenital duplication are regarded as monochorionic monoamniotic[13].

The nomenclature used to describe congenital duplication is based on the point of attachment as discussed by Potter and Craig (1975). When animals are joined at the thorax or near the sternal region and facing one another, this is known as *thoracopagus* as was seen in this case. When animals are joined at the thorax but are back-to-back, this is known as *pygopagus*. Interestingly, Tansel and Yazicioglu (2004) demonstrated that cardiac abnormalities are observed more frequently in parapagus than in other types of congenital duplication as was observed in this case. Twins that are connected at the head are described as *craniopagus*. Lastly, those twins that are joined at the pelvis are known as *ischiopagus*. Additionally, the number of crania, thoracic and pelvic limbs are also described. Dicephalic refers to the presence of two crania and tetrabrachius meaning the presence of four thoracic limbs. As stated earlier, the calves in this case are described, using this nomenclature, as dicephalic, thorocopic, tetraspusal, tetrabrachial.

A variety of etiologies have the potential to induce twinning and congenital abnormalities in livestock, but they are not linked to congenital duplication. Conjoined twins are a nonheritable characteristic and result from either teratogens or are idiopathic in nature[23]. The age of the dam, her parity, and her productivity in lactation have all been shown to be associated with twinning in cattle[10]. The use of ART's has also been shown to increase twinning in cattle[20]. A variety of plants, chemicals and infectious agents (mainly viruses) have been linked to teratogenesis and congenital abnormalities in livestock species [9, 18, 24].

Diagnostics and Treatment

The detrimental health effects associated with conjoined twins in animals are obvious and nearly always result in the death of the neonates in all species. The only exception to this is observed in humans where advanced medical and surgical care can prolong life and even separate conjoined twins in some instances. In cattle specifically, the long-term survival of animals born with congenital duplication is exceedingly rare. Beyond the shortened lifespan, production animals born with congenital duplication are of little-to-no productive value if they survive. Twin calves born without congenital duplication result in high marginal costs to a producer including increased risk of abortion, increased risk of dystocia and subsequent need for veterinary care and therapy, and earlier culling of dams[2, 11].

As discussed previously, twinning occurs in 1-to-7% of births in cattle and occurs more frequently in dairy cattle[10, 22]. It has been previously demonstrated that twin pregnancies are 3.1-to-3.7 times more likely to be terminated early in the fetal period when compared to single calf pregnancies[11, 14]. Furthermore, given that twin pregnancies are more prone to dystocia and neonatal death, it is prudent to determine whether a twin pregnancy, with or without fetal duplication, is present prior to parturition. Rectal palpation and transrectal ultrasound are commonly used to diagnose pregnancy in cattle but only the latter can be used to accurately diagnose congenital malformations early in gestation[6].

Ultrasound is used in human medicine to detect the presence of congenital duplication and is used in the surgical planning process prior to birth[3]. This modality is often employed in veterinary medicine for the diagnosis of pregnancy and could be used to determine the presence of congenital duplication. It can also be used to identify such congenital abnormalities as schistosomus reflexus, and fetal hydrops. In the event that congenital duplication was discovered, termination of the pregnancy is likely the best course of action to prevent the loss of the dam given the progeny will almost certainly die. Ultrasound can be used to facilitate ultrasound-guided reduction of one member of a twin[6].

If a twin pregnancy makes it to term and parturition is imminent, given the risk of dystocia, possible uterine tears and mortality, surgical intervention is almost always necessary[3]. Such a pregnancy has certain surgical considerations that should be made to decrease the risks of morbidity and mortality to the neonates and dam[19]. Dystocia in the case of twins is usually caused by fetal malpresentation and not by the size of the calves as twins are generally smaller when comparted to singleton births[10].

If surgical intervention is required in the event of a twin pregnancy, with or without congenital duplication, the surgeon's primary reason for choosing a particular approach is to minimize the contamination of the abdomen with uterine contents. This is of paramount concern if the fetus is dead and emphysematous as the risk for peritonitis and death of the dam is substantially higher. As such, exteriorizing the uterus to prevent abdominal contamination is extremely important. In this situation, a recumbent ventral midline celiotomy is generally the preferred approach. This approach requires assistance to cast the animal if she is not already recumbent and also to move the animal's legs while the fetus is being removed from the uterus. Furthermore, this approach may make abdominal closure more difficult. This approach is also useful in dairy breeds with large udders and in animals destined for slaughter as damage to the retail cuts is less likely[19].

Conclusion

Twinning is a characteristic that is relatively common in cattle and other ruminants. Congenital duplication occurs much less frequently and is nearly always fatal. The exact mechanism for congenital duplication is somewhat contested but occurs by either fusion or fission of the embryonic discs. Various toxic plants, chemicals, radiological events, and viral etiologies can lead to twinning and congenital duplication in animals and humans. Surgical intervention is nearly always necessary. Certain diagnostic modalities, particularly ultrasonography, can be used to diagnose congenital duplication but their use is often not cost effective given the grave prognosis of the fetuses.

Literature Cited

1. Andreu-Vázquez, C., Garcia-Ispierto, I., López-Béjar, M., de Sousa, N. M., Beckers, J. F., and López-Gatius, F. (2011). Clinical implications of induced twin reduction in dairy cattle. *Theriogenology*, 76, 512-521.
2. Beerepoot, G. M., Dykhuzien, A. A., Nielen, M., and Schukken, Y. H. (1992). The economics of naturally occruing twinning in dairy cattle. *Journal of Dairy Science*, 75, 1044-1051.

3. Cuillier, F., Dillon, K. C., Grochal, F., Scemama, J. M., Gervais, T., Cerekja, A., and Piazze, J. (2012). Conjoined twins: what ultrasound may add to management. *Journal of Prenatal Medicine*,6(1), 4-6.

4. Elnady, F., and Sora, MC. (2009). Anatomical exploration of a dicephalous goat kid using sheet plastination (E12). *Congenital Abnormalities*, 49, 66-70.

5. Gangwar, A. K., Devi, K. S., Singh, A. K., Katiyar, N., Patel, G., and Srivastava, S. (2014). Congenital anomalies and their surgical correction in ruminants. *Advances in Animal and Veterinary Sciences*, 2(7), 369-376.

6. Ginther, O. J. (2014). How ultrasound technologies have expanded and revolutionized research in reproduction in large animals. *Theriogenology*, 81, 112-125.

7. Hiraga, T., and Dennis, S. M. (1993). Congenital duplications. *Veterinary Clinics o North America: Food Animal Practice*, 9, 145-161.

8. Hall, J. G. (2003). Twinning. *The Lancet*, 362: 735 – 743.

9. James, L. F., Panter, K. E., Gaffield, W., and Molyneux, R. J. (2004). Biomedical applications of poisonous plant research. *Journal of Agricultural and Food Chemistry*, 52, 3211-3230.

10. Komisarek, J., and Dorynek, Z. (2002). Genetic aspects of twinning in cattle. *Journal of Applied Genetics*, 43(1), 56-68.

11. Lombard, J. E., Garry, M. B., Tomlinson, S. M., and Garber, L. P. (2007). Impacts of dystocia on health and survival of dairy calves. *American Journal of Dairy Science*, 90, 1751-1760.

12. Lonergan, P., Fair, T., Forde, N., and Rizos, D. (2016). Embryo development in dairy cattle. *Theriogenology*, 86, 270-277.

13. Logrono, R., Garcia-Lithgow, C., Harris, C., Kent, M., and Meisner, L. (1997). Heteropagus conjoined twins due to fusion of two embryos: Report and review. *American Journal of Medical Genetics*, 73, 239 – 243.

14. López-Gatius, F., and Hunter, R. (2005). Spontaneous reduction of advanced twin embryos: its occurrence and clinical relevance in dairy cattle. *Theriogenology*, 63, 118-125.

15. Peippo, J., Machaty, Z., and Peter, A. (2011). Terminologies for the pre-attachment bovine embryo. *Theriogenology*, 76, 1373-1379.

16. Potter, E. L., & Craig, J. M. (1975). Pathology of the Fetus and the Infant. Chicago: Book Medical.

17. Purohit, G. N., Kumar, P., Solanki, K., Shekher, C., and Yadav, S. P. (2012). Perspectives of fetal dystocia in cattle and buffalo. *Veterinary Science Development*, 2(8), 31-42.

18. Samuel M. O., Wachida, N., Abenga, J. H., Kisani, A. I., Adamu, S. S., Hambesha, P., Gyang, E., and Oyedipe, E. O. (2014). A case of omphalo-ischiopagus (dicephalic dithoracic abdominopagus tetrascelus tetrabrachius) in lambs. *Anatomia Histologia Embryologia*, 43(4), 320-323.

19. Schultz, L. G., Tyler, J. W., Moll, D., Constantinescu, G. M. (2008). Surgical approaches for cesarean section in cattle. *Canadian Veterinary Journal*, 49, 565-568.

20. Schneevoigt, J., Bahramsoltani, M., Gerlach, K., Gericke, A., Fersterra, M., Brehm, W., and Seeger, J. (2014). Aarapagus conjoined twin calf: a case study – Focused on CT and cardiac abnormalities. *Anatomia Histologia Embryologia*, 43, 64-70.

21. Senger, P. L. (2003). Pathways to Pregnancy and Parturition (2nd ed.). Ephrata, PA: Cadmas Professional Communications.

22. Silvia-del-Rio, N., Colloton, J. D., and Fricke, P. M. (2009). Factors affecting pregnancy loss for single and twin pregnancies in a high-producing dairy herd. Theriogenology, 71, 1462-1471.

23. Singh, G., and Pandey, K. (2013). Dystocia due to conjoined twin monsters in Murrah Buffaloes. *Haryana Veterinarian*, 52, 139-140.

24. Smolec, O., Kos, J., Vnuk, D., Stejskal, M., Brkljaca, B., and Zobel, R. (2010). Multiple congenital malformation in a Simental female calf: a case report. *Veterinarni Medicina*, 55 (4), 194-198.

25. Spencer, R. (1992). Conjoined twins: Theoretical embryologic basis. *Teratology*, 45, 591-602.

26. Spencer, R. (2001). Theoretical and analytical embryology of conjoined twins: Part I: Embryogenesis. *Clinical Anatomy*, 13, 36-53.

27. Spitz, L., and Kiely, E. M. (2003). Conjoined twins. *Journal of the American Medical Association*, 289(10), 1307-1310.

28. Tansel, T., and F. Yazicioglu. (2004). Cardiac and other malformations in parapagus twins. *Archives of Gynecology and Obstetrics*, 269, 211–213.

29. Vanderzon, D. M., Partlow, G. D., Fisher, K. R. S., and Halina, W. G. (1998). Parapagus conjoined twin Holstein calf. *The Anatomical Record*, 251, 60-65.