J Vet Diagn Invest 11:368–371 (1999)

Multiple congenital anomalies in a calf

S. J. Newman, T. L. Bailey, J. C. Jones, W. A. DiGrassie, W. D. Whittier

A 4-hour-old mixed-breed black heifer calf produced from *in vitro* fertilization and born via Caesarian section 6 days prior to the anticipated due date was euthanized because of multiple obvious congenital anomalies and an inability to stand. The number of congenital anomalies was remarkable and included anal and vulvar atresia, hypoplastic clitoral tissue, cervical block vertebrae, anotia, brachygnathia inferior, secondary cleft palate, calvarial doming with hydrocephalus and stenosis of the Sylvian aqueduct, high ventricular septal defect, rectovaginal fistula, and dermal hamartomas.

Few previous reports of congenital anomalies in calves have documented the number and the severity of those seen in this calf. Because development of tissues and organs is interdependent, it is not uncommon for more than 1 anomaly to appear in an animal. In this calf, several rarely reported anomalies, such as anotia, vulvar atresia, and dermal hamartomas, were evident; these anomalies have not been previously documented in cattle.

An oocyst from an apparently healthy slaughterhouse cow was fertilized with sperm from a transgenic bull and implanted in a 16-month-old recipient heifer on day 8 of embryo development. Gestation proceeded without any clinical evidence of difficulty. The dam was vaccinated[a] against infectious bovine rhinotracheitis, parainfluenza virus, bovine viral diarrhea virus, bovine respiratory syncytial virus, and 5 strains of leptospires (canicola, grippotyphosa, hardjo, icterohaemorrhagiae, pomona) and treated with an anthelmintic[b] 60 days prior to utilization as the recipient dam. An *Escherichia coli*, bovine coronavirus, and rotavirus vaccine was administered 8 weeks and 4 weeks prior to the anticipated parturition date.[c] A second injection of anthelmintic was also administered 8 weeks prior to the expected parturition.

This calf was euthanized because of the presence of anal and vulvar atresia, which are incompatible with life. Necropsy results confirmed the presence of additional external anomalies for this small-term calf, including hypoplastic clitoris, meconium-filled perivulvar subcutaneous sac, short neck, aplasia of the external, middle, and inner ear canals (anotia), brachygnathia inferior, secondary cleft palate, calvarial doming, and locally extensive alopecia over the dorsal cranium.

Internal congenital anomalies included stenosis of the Sylvian aqueduct resulting in severe bilateral congenital internal hydrocephalus (Fig. 1), bilateral agenesis of the tympanic bullae, a large and high ventricular septal defect, bilateral hypoplastic ovaries (right, 1.5 cm long; left, 1.7 cm long), and a rectovaginal fistula (Fig. 2) associated with the imperforate anus and vulva. This fistula had resulted in the production of a meconium-filled cloaca, which protruded externally as a pendulous subcutaneous sac containing rudimentary clitoral tissue at its apex. Additionally, the rectovaginal fistula and accompanying cervical hypoplasia allowed for meconium to greatly distend the left uterine horn. The right uterine horn, although present, was small and discontinuous with the left because of segmental aplasia.

Block vertebrae were identified at C3-4, C6-7, and T1-2. Fusion occurred primarily between the vertebral arches at C3-4 and C6-7 and involved both the vertebral bodies and the arches at T1-2. The vertebral arches of C5 and C6 were only partially formed. The C5 vertebra was angled dorsally relative to the C4 vertebra (Fig. 3).

The following tissues were collected into 10% buffered formalin for histologic examination: lymph node, small intestine, heart, liver, spleen, skin, ear cartilage, ovary, thyroid gland, rumen, inguinal fat, pancreas, lung, adrenal gland, kidney, eye, cervical spinal cord, and brain. Hepatocellular vacuolation with retained central placement of the nucleus consistent with glycogen accumulation was noted. Neutrophils were prominent within the splenic parenchyma. Eosinophil numbers were increased within the lamina propria of the small intestinal villi. There was multifocal alveolar hemorrhage. Several fetal glomeruli were identified in the renal cortex. Hair follicle and adnexal agenesis was present in the alopecic areas over the calvarium. Epidermal thinning and decreased surface keratinization in association with the presence of a localized proliferation of dysplastic bilayered columnar glandular structures (resembling apocrine gland) among adipose tissue and immature dermal fibroblasts were indicative of a dermal hamartoma (Fig. 4). The gonads were ovarian but failed to reveal development of tertiary ovarian follicles. Small numbers of secondary follicles and larger numbers of both primary and primordial follicles were identified. Expanded lateral ventricles were lined by flattened ependymal epithelium that focally had lost apical cilia. Stenosis of the Sylvian aqueduct was identified in the midbrain. Within the cerebral cortex at the level of the basal ganglia, there was a disorderly arrangement of neurons, particularly within the deep laminar layers, such that clusters and small nodules were present. Individual neurons appeared fully differentiated.

The cause of the multitude of developmental anomalies in this calf could not be determined. Many of these anomalies in the bovine population have been associated with genetic factors (transgenes, chromosomes), environmental agents (infections, toxins, fertilization techniques, management), or a combination of factors. The mechanism(s) by which the multiple anomalies arose in this case is open for speculation. This calf was not transgenic despite fertilization with semen from a transgenic bull. Unfortunately, the genetic karyotype samples were nondiagnostic, and numerical chromosomal abnormalities could not be entirely excluded. Similarly, be-

From the Departments of Biomedical Sciences and Pathobiology (Newman), Large Animal Clinical Sciences (Bailey, DiGrassie, Whittier), and Small Animal Clinical Sciences (Jones), Virginia–Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0442.

Present address: Animal Health Laboratory, Laboratory Services Division, University of Guelph, Box 3612, Guelph, Ontario, Canada N1H 6R8 (Newman).

Received for publication July 14, 1998.

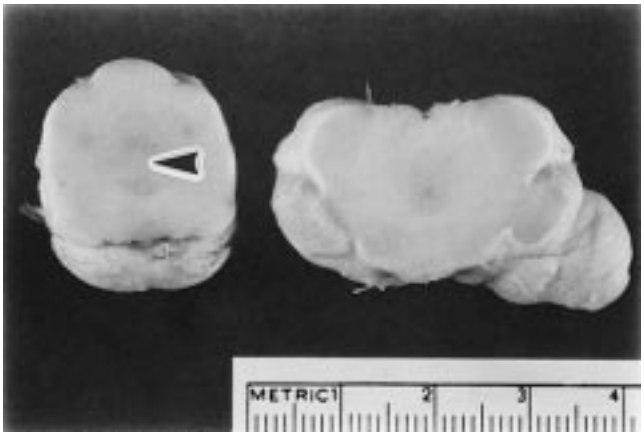

Figure 1. Medulla; calf. Stenosis of the Sylvian aqueduct (arrowhead) producing internal hydrocephalus at the level of the fourth ventricle.

cause the oocyst had been harvested from a slaughterhouse cow, the contribution of maternal chromosomal abnormalities was unknown. The recipient heifers were routinely vaccinated for teratogenic viral agents, particularly bovine viral diarrhea virus (BVDV),[a] 60 days prior to embryo transfer and 2 months prior to parturition with a killed BVDV vaccine.[d] All heifers were also screened for BVDV via virus isolation prior to entering the herd farm. Bovine viral diarrhea had not been a clinical problem on this farm, and it had not been implicated as a cause of abortion in other fetuses previously submitted for diagnostic evaluation. Access to toxic agents (organic, metallic, chemical) was also considered unlikely.

Calves produced by in vitro fertilization are often larger at birth than naturally bred calves because of the techniques of embryo culture and the manipulation of preimplantation embryos.[6,27] Increased rates of abortion, physical abnormalities (particularly involving the skeletal system), and mortality have been documented in offspring produced by *in vitro* fertilization.[6,27] Because cattle embryos do not undergo transcription until the 8–16-cell stage rather than the 2-cell stage as in mice, the longer culture periods have a more substantial effect on gene function.[27] Additionally, high serum content in the culture medium has been implicated in cytoplasmic fragmentation and resultant deleterious effects on embryo development.[27] Serum components may have a direct effect on the genome or may act indirectly by affecting the integrity of cytoplasmic organelles and membranes.[27] Bovine serum albumin and amino acids in the medium as suggested to produce animals with normal birth weights was used in this case to minimize these effects.[27]

Frequent (twice weekly from day 28 to day 90 of gestation) rectal ultrasound examinations may have contributed to the development of atresia ani. Intestinal atresia is thought to occur following disruption of vascular integrity and has been associated with rectal palpation for pregnancy diagnosis before day 42 of gestation.[2,5]

An estimated 0.5–1.0% of calves born have spontaneous congenital defects,[12] and a higher percentage succumb to abortion.[1] Fetal losses, reduced gestation length and birth weight, incidences of dystocia, perinatal loss, and anomalies have been greatest for embryos produced by in vitro procedures and nuclear transfer.[10,29] In 1 study, calves produced by in vitro procedures had increased occurrences of heart failure, double muscling, hydroallantois, leg and joint problems, larger than normal organs, and cerebellar dysplasia.[10] Abnormal growth of organs and skeletal elements is thought to occur more frequently when there is asynchrony between the recipient and the developmental stage of the embyro.[10]

Multiple congenital malformations may occur because malformation of 1 portion of the body directly leads to mal-

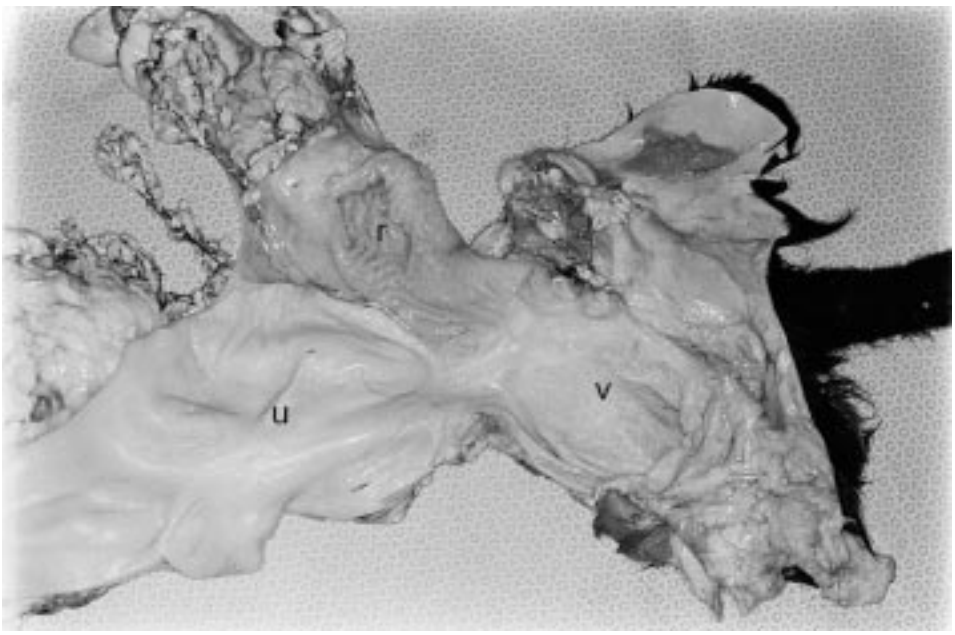

Figure 2. Rectum (r), uterine horn (u), and vagina (v); calf. Note communication between the rectum and vagina.

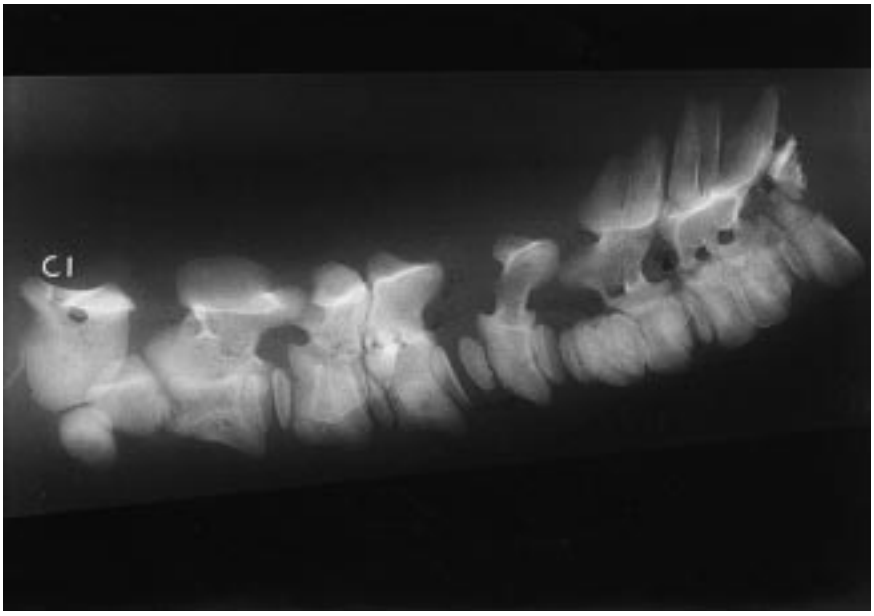

Figure 3. Cervical spine; calf. Lateral radiographs of sagittal slices. Block vertebrae are identified at C3-4, C6-7, and T1-2. Vertebral arches of C5 and C6 were only partially formed. C5 vertebra is angled dorsally relative to the C4 vertebra.

formation of others.[1] Chromosomal alterations typically lead to anomalous syndromes rather than single malformations.[1] Additionally, teratogens may act simultaneously or successively upon various tissues during development.[1]

This calf was unique because of the extent of multisystem malformation. Combinations of several similar defects have been reported. Atresia ani occurs as an isolated defect but is more commonly seen in combination with other intestinal malformations.[4] Ventricular septal defects are the most common cardiovascular anomaly in calves and may occur alone or in association with other cardiac anomalies[17,19,20,28] and with hydrocephalus.[19] Internal hydrocephalus can occur concurrently with multiple ocular defects, myopathy, or arthrogryposis in calves.[12] Cleft palate may occur singly, but in Charolais and Hereford cattle, a recessively inherited syndrome of arthrogryposis and palatoschisis exists.[14,19]

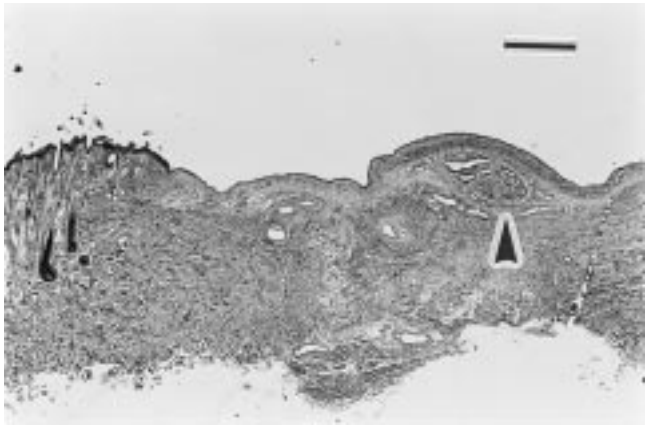

Figure 4. Skin; calf. Normal dermal adnexal structures are absent and replaced by a dysplastic proliferation of glandular elements consistent with a dermal hamartoma (arrowhead). HE. Bar = 800 μm.

Congenital anomalies in offspring of natural breedings are often a result of environmental factors, genetic factors, or both. Environmental factors are implicated in internal hydrocephalus (exposure to toxic plants, viruses, drugs, trace elements, irradiation, hyperthermia, and excessive pressure on the amniotic vesicle during early rectal pregnancy examination[2,5,12]), cleft palate (secondary to xenobiotic effects[19]), arthrogryposis (exposure to toxic plants [lupines, hemlock], viral infections, or nutritional deficiencies [manganese]), and anotia (in human infants following ingestion of thalidomide).[8,16]

A genetic basis has been documented for some cases of atresia ani, but the specific cause in sporadic cases in domestic species and humans is not always known.[9,15,21] Similarly, a genetic basis has been determined for internal hydrocephalus (simple autosomal recessive trait in many cattle breeds and a familial condition in Charolais calves[11–13]), cleft palate (multifactorial or autosomal inheritance in the Charolais breed[19]), brachygnathia inferior (polygenic inheritance in the Simmental breed[7,30]), ventricular septal defect (autosomal dominant trait), arthrogryposis, and rectovaginal fistula (in 1 study, chromosomal evaluation failed to reveal associated numerical abnormalities).[24]

A summary of the proposed pathogenesis of some of the more unique anomalies follows. Failure of the anal membrane to perforate, failure of the bowel to canalize, failure of the proctodeum to invaginate, and interruption of the blood supply to the anus or to the intestine during embryonic development can produce atresia ani or intestinal atresia, respectively.[2,4,5,9,18] Atresia ani may develop when the dorsal part of the cloacal plate fails to form,[21] and in females this is occasionally accompanied by a rectovaginal fistula.[3] The resulting fistula connects the dorsal wall of the vagina with the ventral portion of the terminal rectum and provides a path for defecation.[23] Defecation is inhibited when atresia of

the vulva accompanies this lesion. Atresia of the vulva has not been previously reported in calves.

Block vertebrae result from a failure of segmentation. One proposed mechanism for the problem is malformation of the intersegmental arteries in the developing embryo.[25]

Hamartomas are defined as congenital malformations that present as masses of disorganized tissue indigenous to the particular anatomic site. In this calf, dysplastic apocrine glands proliferated within a focal area of dermal collagen maturational arrest. Other adnexal structures such as sebaceous glands and hair follicles were absent. This is the first report of dermal hamartomas in calf skin.

Anotia (the absence of ears) has not previously been reported in calves. In human beings, ear anomalies are divided into 2 forms; minor anomalies restricted to the middle ear and major anomalies including additional malformations of the external meatus and, less frequently, the auricle.[26] Complete absence of development of the inner ear with aplasia of the labyrinth, accompanied by narrowing of the middle ear and a normal appearance to the outer ear and tympanic membrane is a rare condition referred to as Michels type anomaly in human beings.[16,22] In this calf, the tympanic bulla, inner ear, middle ear, and external auditory canal were absent and only rudimentary bilateral cartilaginous skin flaps were identified.

This case report includes descriptions of several unique features in the pathology of bovine fetal malformation and is one of the first to report vulvar atresia, anotia, and dermal hamartomas in a calf. Additionally, the extent and multitude of malformations in this live-born calf, with a total of 14 separate congenital abnormalities affecting 6 different organ systems, is remarkable. The cause(s) of these abnormalities could not be determined, but viral agents and toxins seemed unlikely. The production of this calf using *in vitro* fertilization was considered a possible cause. Because the calf was not a transgenic animal, insertional genetic defects also were considered unlikely.

Acknowledgements. We gratefully acknowledge the help of Mr. Jerry Baber, Biomedical Media, VA-MD Regional College of Veterinary Medicine for assistance with the photographs. Dr. Stephen A. Smith, DVM, PhD, is acknowledged for editorial assistance.

Sources and manufacturers

a. Herd Vac 9®, Bayer, Shawnee Mission, KS.
b. Ivomec®, Merck and Co., Rahway, NJ.
c. Scour Guard 3®, Smith Kline Beecham, Exton, PA.
d. Horizon 10®, Bayer, Shawnee Mission, KS.

References

1. Camon J, Sarate D, Franch J, et al.: 1990, Associated multiple congenital malformations in domestic animals. Contribution of four cases. J Am Vet Med Assoc 37:659–668.
2. Constable PD, Rings DM, Hull BL, et al.: 1989, Atresia coli in calves: 26 cases (1977–1987). J Am Vet Med Assoc 195:118–123.
3. Cooke WD: 1981, Atresia ani and atresia of the external genitalia in a pig. Vet Med Small Anim Clin 76:1037–1038.
4. Dennis SM, Leipold HW: 1972, Atresia ani in sheep. Vet Rec 91:219–222.
5. Dreyfuss DJ, Tulleners EP: 1989, Intestinal atresia in calves: 22 cases (1978–1988). J Am Vet Med Assoc 195:508–513.
6. Farin PW, Farin CE: 1995, Transfer of bovine embryos produced in vivo or in vitro: survival and fetal development. Biol Reprod 52:676–682.
7. Griffith JW, Hobbs BA, Manders EK: 1987, Cleft palate, brachygnathia inferior and mandibular oligodontia in a Holstein calf. J Comp Pathol 97:95–99.
8. Isenberg SF, Tubergen LB: 1979, Unilateral complete aplasia of the inner ear with associated tracheoesophageal fistula: report of a case. Otolaryngol Head Neck Surg 87:435–439.
9. Johnson EH, Nyack B, Aston M: 1980, Atresia ani and rectovaginal fistula in a goat. Vet Med Small Anim Clin 75:1833–1835.
10. Kruip TAM, den Daas JHG: 1997, In vitro produced and cloned embryos: effects on pregnancy, parturition and offspring. Theriogenology 47:43–52.
11. Leech RW, Haugse CN, Christoferson LA: 1978, Hydrocephalus: congenital hydrocephalus. Am J Pathol 92:567–571.
12. Leipold HW, Dennis SM: 1987, Congenital defects of the bovine central nervous system. Vet Clin North Am Food Anim Pract 3:159–177.
13. Leipold HW, Hibbs CM: 1974, Bovine congenital defects: variations of internal hydrocephalus. Cornell Vet 64:596–616.
14. Leipold HW, Huston K, Hulbert LC, et al.: 1974, Congenital syndrome in hereford calves with kyphoscoliosis, arthrogryposis and palatoschisis. Cornell Vet 64:123–125.
15. Lindner VH, Kiesel L, Woitek G: 1988, Isolated occurrence of a congenital rectovaginal fistula in a girl. Zentralb Chir 113: 1580–1582.
16. Lindsay JR: 1971, Inner ear histopathology in genetically determined congenital deafness. Birth Defects 7(4):21–32.
17. Mclennan MW, Sutton RH: 1993, Ventricular septal defect and an atrioventricular valvular anomaly in a heifer. Aust Vet J 70: 425–426.
18. Prieur DJ, Dargatz DA: 1984, Multiple visceral congenital anomalies in a calf. Vet Pathol 21:452–454.
19. Rousseaux CG: 1994, Congenital defects as a cause of perinatal mortality of beef calves. Vet Clin North Am Food Anim Pract 10:35–45.
20. Sandusky GE, Smith CW: 1981, Congenital cardiac anomalies in calves. Vet Rec 108:163–165.
21. Saperstein G: 1993, Congenital abnormalities of internal organs and body cavities. Vet Clin North Am Food Anim Pract 9:115–125.
22. Subotic R, Schuster E: 1978, Simultaneous histological, audiological and radiological findings in congenital anomalies of the inner ear. J Laryngol Otol 92:281–291.
23. Suess RP, Martin RA, Moon M, et al.: 1992, Rectovaginal fistula with atresia ani in three kittens. Cornell Vet 82:141–153.
24. Swartz A, Vogt DW, Michalski KA, et al.: 1985, Chromosomal evaluation of a ewe lamb with atresia ani vaginalis. Am J Vet Res 46:2145–2146.
25. Tanaka T, Uthoff HD: 1981, The pathogenesis of congenital vertebral malformations. Acta Orthop Scand 52:413–425.
26. Teunissen EB, Cremers WRJ: 1993, Classification of congenital middle ear anomalies. Report on 144 ears. Ann Otol Rhinol Laryngol 102:606–612.
27. Walker SK, Hartwich KM, Seamark RF: 1996, The production of unusually large offspring following embryo manipulation: concepts and challenges. Theriogenology 45:111–120.
28. West HJ: 1988, Congenital anomalies of the bovine heart. Br Vet J 144:123–130.
29. Willadsen SM, Janzen RE, McAlister RJ, et al.: 1991, The viability of late morulae and blastocysts produced by nuclear transplantation in cattle. Theriogenology 35:161–170.
30. Woollen NE: 1993, Brachygnathia in Simmental cattle. Agric Res Serv 71:183.