

Case report

Bulldog calf born to Limousin-Peranakan-Ongole crossbred cow after insemination with Simmental bull semen

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ABSTRACT

This case describes a bulldog calf born to Limousin-Peranakan-Ongole (LimPO) crossbred cow after insemination with frozen-thawed Simmental bull semen. The dam was a four year old LimPO crossbred cow, with a bodyweight of 380 kg; she was 8.5 months pregnant and has never had any reproductive disorders before. Signs of impending calving appeared at 6 am, and the discharge was normal, but until noon the calf had not been born. Body temperature was 38°C, while other vital signs were normal. Vaginal exploration revealed a completely opened cervix, and the calf was alive, in posterior longitudinal presentation with palpable short hindlegs. The calf was delivered per vaginam with traction. The calf was male with a weight of 15 kg, and was alive, but after a few minutes, the calf died. The monstrous calf was found with morphological defects including a smaller compressed body, head resembled those of a bulldog with a short muzzle and an undershot jaw, shortened fore- and hindlegs, and ascites abdomen. The dam was given 70, 000 IU procaine penicillin G and 70 mg dihydrostreptomycin sulphate (Penstrep-400, Interchemie, Holland), 0,3 g diphenhydramine HCl (Vetadryl, Sanbe, Indonesia), 10 mg vitamin B12 (Jectavit B12, Sanbe, Indonesia) by intramuscular injection. The dam looked healthy, and had normal appetite after the delivery assistance.

Keywords: Bulldog calf, Limousin-Peranakan-Ongole crossbred, molecular genetics, pattern of inheritance, Simmental bull semen

INTRODUCTION

Congenital malformations are morphological defects that develop during intrauterine life and could be observed at birth (Uzar *et al.*, 2020). Such developmental disorders resulted from errors in the complex events of organogenesis. The causes of congenital malformations were genetic, environmental, and specific infections during fetal development (Adeyemi and Aina, 2019). Dwarfism is one of the most popular defects in

beef cattle. Dwarfism can be grouped into proportional and disproportionate dwarfism (Ciepluch *et al.*, 2017). Proportional dwarfism also called pituitary or ateliotic dwarfism was characterized by small but proportionally sized extremities. Proportional dwarfism was caused by disruption of the hypothalamic-growth hormone-insulin-like growth factor axis, followed by reduced bone and soft tissue formation, resulting in an infantile phenotype. Disproportionate dwarfism is also called defect chondrodysplasia, chondrodystrophy, and

achondroplasia, which is characterized by limb disproportion (rhizomelia), skull deformity (flat and broad head), endochondral ossification defects, and an excessive amount of soft tissue (Murgiano *et al.* 2014).

Phenotypically, chondrodysplasia varied widely, ranging from lethal fetal abnormalities and semi-lethal to the calf being born and surviving on short legs. Chondrodysplasia is generally a genetic factor (Moura *et al.*, 2014). The inheritance pattern of chondrodysplasia also varies between different phenotypes and breeds. There are several genetic pathways involved in chondrodysplasia, resulting in abnormal function. Pathogenetic chondrodysplasia is characterized by impaired endochondral osteogenesis (Agerholm *et al.*, 2004).

Bulldog dwarfism malformations in cattle were first reported in Dexter cattle in the 18th century (Cavanagh *et al.*, 2007). Meanwhile, the first more complete description of chondrodysplasia in cattle was published in 1904. Chondrodysplasia harms the development of the entire skeleton resulting in the disproportion between skeletal segments (Moura *et al.*, 2014). Chondrodysplasia is one of the rare congenital anomalies in cattle, buffalo, and sheep (Prasad *et al.*, 2016). Bulldog calf cases could occur in pure breed Holstein (Agerholm *et al.*, 2004), Jersey, Nellore cattle (Moura *et al.*, 2014), Angus breed cattle (Latter, 2006), Italian Tyrolean Gray cattle (Murgiano *et al.*, 2014), American Angus, Hereford and Ayrshire cattle (Koltjes *et al.*, 2009). Bulldog calf cases could also be found in Simmental cross breeds, Hereford-Angus, South Devon-Angus (Blowey and Weaver, 2011), and Holstein-Jersey crossbreeds. So far, there have been no reports of bulldog calves born from the Limousin-Ongole crossbreed cow. This case report discusses a calf with congenital defects which was born to a Limousin-Ongole crossbred cow artificially inseminated with frozen-thawed semen of Simmental bull. Inheritance patterns and molecular genetics are discussed to prevent similar occurrences in the future.

METHODS

This case occurred in Balak Hamlet, Pendoworejo urban-village, Girimulyo sub-district, Kulon Progo regency, Yogyakarta

special region province, on November 29, 2021. Geographically, Kulon Progo regency is located between 7°38'42" - 7°59'3" South Latitude and 110°1'37"- 110°16'26" East Longitude. The dam, in this case, was a four year old Limousin-Peranakan-Ongole (LimPO) crossbred cow, with a bodyweight of 380 kg; she was 8.5 months pregnant and has never had any reproductive disorders before. The feed given was kolonjono grass, field grass, grass straw, and pollard bran, and drinking water was always available in the cage.

Anamnesis, physical examination, and treatment

Signs of impending calving appeared at 6 am, characterized by normal vaginal discharge, but until noon the calf had not been born, and finally, the owner called the veterinarian. Eating and drinking was normal; physical examination showed a body temperature of 38°C, normal breath, shiny hair, regular stool, normal pulse, normal facial expression, wet and warm nose. Examination by vaginal exploration revealed a completely open cervix, and the calf was in a posterior longitudinal presentation with palpable short hind legs. Anal sphincter reflex and umbilical pulse, indicated that the fetus was alive. The calf was delivered per vaginam with traction; and then, the dam was treated with 70,000 IU procaine penicillin G and 70 mg dihydrostreptomycin sulphate (Penstrep-400, Interchemie, Holland), 0,3 g diphenhydramine HCl (Vetadryl, Sanbe, Indonesia), 10 mg vitamin B12 (Jectavit B12, Sanbe, Indonesia) by intramuscular injection.

RESULTS

The calf was successfully expelled by traction. The calf was male with a weight of 15 kg, and was alive, but after a few minutes, the calf died. The monstrous calf was found with defects including a smaller compressed body (Figure 1 A), head resembled those of a bulldog (Figure 1 B) with a short muzzle and an undershot jaw (Figure 1 C), shortened fore- and hindlegs (Figure 1 D and E), and ascites abdomen (Figure 1 E). The dam looked healthy, and had normal appetite.



Figure 1 Achondroplastic fetus born with some morphological defects; smaller compressed body (A), head resembled those of a bulldog (B), with a short muzzle and an undershot jaw (C), shortened fore- and hindlegs (D and E), and ascites abdomen (E).

DISCUSSION

The calf's birth weight in this case (15 kg) was very low compared to the average birth weight of the LimPO calf, which was 34.77 ± 1.97 kg (Susanti *et al.*, 2015). In disproportionate dwarfism calves, the body was short due to reduced axial skeleton length, with an average length of only about 55 cm. The fore- and hindlegs showed bilateral symmetrical

malformations with a shorter size than the average calf, but the digits were relatively normal. Pathoanatomically in the Bulldog calf, the most prominent features were disproportionate body morphology, micromelia of the forelegs and hindlegs, short spine, large abdomen, macrocephaly, and short facial bones with protruding tongue. The head was brachycephaly; the dorsal skull was reduced craniocaudal and increased laterally, with prominent frontal and parietal regions (Dittmer *et al.*, 2017).

The trachea was stenotic at the cranial thoracic aperture, the ribs are short, causing narrowing of the thoracic cavity and compression and hypoplasia of the lungs (Windsor *et al.*, 2006). Compression of the lungs with the lungs not filled with air (Agerholm *et al.*, 2004) would be followed by dyspnea in the first days of life, leading to death (Blowey and Weaver, 2011). The heart was spherical with bilateral ventricular hypertrophy and decreased ventricular volume. The large and protruding abdomen was due to the normal volume of the viscera but the very short lumbar spine (Moura *et al.*, 2014).

Inheritance pattern

Achondroplastic dwarfism could be caused by genetic factors (chromosomal or gene mutation) or environmental factors (chemical or physical teratogenicity, infection, etc.), or a combination thereof (Uzar *et al.*, 2020). Bulldog calf syndrome was congenital anomalies resulting from Mendelian inheritance from the parents (Prasad *et al.*, 2016). Several bulldog calves have shown that the inheritance of chondrodysplasia in the Dexter breed followed a homozygous dominance mechanism. In simple terms, the inheritance pattern of Bulldog calves could be illustrated as in Figure 2. If the symbol for the allele "a" and the mutant allele was "A", long-legged Dexter cattle had a genotype of "aa" (homozygous), and short-legged Dexter cattle had a genotype of "Aa" (heterozygous) and the bulldog Dexter had the genotype "AA" (homozygous) (Moura *et al.*, 2014).

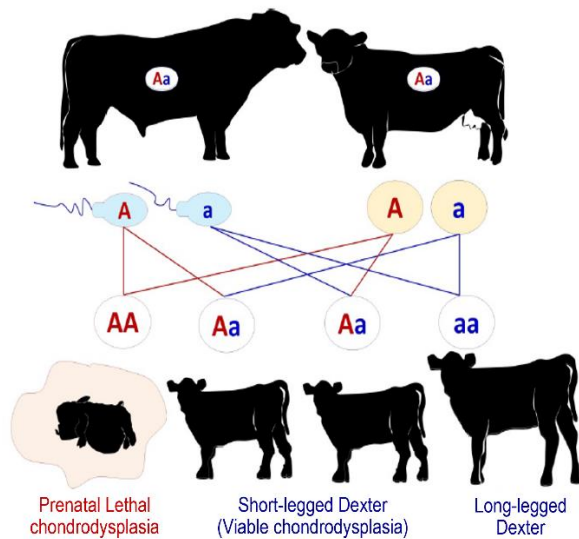


Figure 2 Dwarfism inheritance pattern based on Mendel's Law (Moura *et al.*, 2014).

In addition, there was a genetic model involving unstable mutants with high frequency in the dominant dwarfism allele expressed during gametogenesis (Latter, 2006). This genetic inheritance could be autosomal recessive or incompletely autosomal dominant. Whitlock *et al.* (2008) reported that cows with a heterozygous genotype have this defect, while genotypically homozygous individuals were aborted or die soon after birth.

Dwarfism Molecular Genetics

Molecularly, there are three types of dwarfism, namely proportional dwarfism with inflammatory lesions, Aggrecan type dwarfism, and Chondrodysplasia (Cieplach *et al.*, 2017). Proportional dwarfism was caused by mutations in the RING finger protein 11 (RNF11) gene located on chromosome three. The causative mutation was the transition of the DNA coding gene from Adenine to Guanine (c.124_2A>G) and changed the intron acceptor site of one of the genes. RNF11 encoded the finger protein RING 11—a subunit of the ubiquitin A20 editing complex that regulated Nuclear Factor Kappa Beta (NF-) signaling. Proteins paid for due to mutations caused loss of function, so tissue growth was inhibited. This mutation could be detected by quantitative reverse transcription-polymerase chain reaction (RT-PCR), 5'-exonuclease assay, and capillary sequencing (Sartelet *et al.*, 2012).

The second type was aggrecan type dwarfism, which caused bulldog dwarfism and was first discovered in Dexter cattle (Cieplach *et al.*, 2017). It was one of the most common forms of disproportionate dwarfism, caused by mutations in the ACAN gene on bovine chromosome 21. The mutation was a 4-bp insertion in exon 11, the base GGCA at a position between the sequences 2266 and 2267 (c.2266_2267insGGCA), and the C to T transition in exon 1 (c.-198C>T), which were called BD1 and BD2, respectively. ACAN encoded aggrecan, an aggregating proteoglycan that was an essential structural component of cartilage. BD1 and BD2 mutations caused a significant reduction or even absence of aggrecan so that cartilage growth was inhibited, which resulted in impaired long bone development (Moura *et al.*, 2014). Stillborn calves with markedly chondrodystrophic dwarfism with hydrocephalus were born from a normal dam with a bovine BD1 heterozygous (Cabrera *et al.*, 2016). ACAN mutation detection could use PCR, PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) with Hsp92I for BD1 and BD2. Confirmation of these techniques by radiography could identify differences between normal cows, sufferers, or carriers of dwarfism (Cavanagh *et al.*, 2007).

The ACAN gene was located in band five regions 1 of the long arm of bovine chromosome 21 (BTA21q15). This gene encoded aggrecan, a protein present in the extracellular matrix of cartilage tissue and was required for the normal formation of bone. The matrix was called chondroitin sulfate proteoglycan core protein 1 (CSPGCP or CSPG1). Different possible causative variants (ACAN:c.5686insC; p.Val1898fsTer9), homozygous dwarfism calves originating from parents and males who were heterozygous, respectively (Struck *et al.*, 2018).

The third type was Chondrodysplasia (Cieplach *et al.*, 2017). Disproportionate dwarfism chondrodysplasia was caused by a mutation in the EVC2 gene on chromosome 6. The causative mutation was a 2-bp deletion (c.2993_2994ACdel) in exon 19 of the Tyrolean Gray bovine gene. The EVC2 gene, also called the LBN gene, encodes the Ellis-van Creveld syndrome 2 protein. The mutation caused a frameshift and premature termination of codons. This mutation resulted in an impaired in the

development of the skull, spine, and other limbs. Radiographic techniques, single nucleotide polymorphism (SNP) array genotyping, and Sanger sequencing made it possible to find these deletions (Muscatello *et al.*, 2015).

In Angus cattle, Dwarfism was disproportionate with mutations in the PRKG2 gene on chromosome 6 (Cieplóch *et al.*, 2017). The product of PRKG2 expression was a type II protein dependent on cGMP kinase as an essential signal transduction pathway for tissue formation and growth of plate cartilage. The causative mutation was the transition from C to T (c.2032C>T) localized at exon 15, leading to premature termination of codons in the kinase domain. These mutations altered the expression levels of COL2 and COL10, which played a crucial role in the growth plate (Koltes *et al.* 2009). Experiments showed that the R678X mutation resulted in a loss of PRKG2 regulation of COL2 and COL10 mRNA expression as the cause of mutations in chondroplastic dwarfism cattle (Koltes *et al.*, 2009). The COL2A1 missense mutation could also cause lethal chondrodysplasia in the offspring of French Holstein males (Agerholm *et al.*, 2016). Bulldog calf syndrome resulted from de novo mutation events that occur either post-zygote in embryos inherited from the dam or the male (Jacinto *et al.*, 2020). The Ellis van Creveld syndrome 2 (EVC2) gene was associated with chondrodysplastic dwarfism in Japanese Brown cattle. Sanger sequencing confirmed the presence of a 2 bp deletion in exon 19 (c.2993_2994ACdel), causing a premature stop codon in the bovine EVC2 coding sequence (Murgiano *et al.*, 2014).

Achondroplasia was a genetic disorder caused by mutations in the Fibroblast Growth Factor 3 (FGFR3) receptor. This mutation caused an increase in chondrocyte inhibitory signals on the growth plate. Recifercept was a potential drug for dwarfism. Recifercept worked by binding to the FGFR3 ligand, which then normalized the activation of the FGFR3 receptor. Recifercept was shown to bind to FGF isoforms in vitro and cellular model systems and attenuate FGFR3 signaling. Moreover, in a transgenic mouse model of achondroplasia, Recifercept restored reduced body weight and long bone growth in these mice (Gonçalves *et al.*, 2020).

CONCLUSION

The rescue of the bulldog calf case born to the Limousin-Peranakan-Ongole crossbred cow after insemination with the frozen-thawed semen of the Simmental bull has been successfully carried out. A male fetus weighed 15 kg, was successfully delivered alive, but after a few minutes, the fetus died. There was an abnormality in the shape of the calf which resembled a bulldog. The dam was healthy, with normal appetite after the delivery assistance. Prevention could be done by rejecting males with a DNA profile carrying the bulldog calf gene.

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