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Atresia Ani, Hypospadia and Rudimentary External Genitalia in Two German Holstein Calves

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Abstract: In the present report, clinical, pathological, and cytogenetic examinations of a one-day-old male German Holstein calf with atresia ani, hypospadia and rudimentary external genitalia (HYP-AA) and a six-months-old male German Holstein calf with hypospadia and rudimentary external genitalia (HYP) are presented. In the first case (HYP-AA), an anal orifice was missing. The urethra opened in the perineum. Consequently, the inguinal region was infiltrated with urine. A secondary finding was a vesicle at the anal area with a diameter of approximately 10 cm. Histopathological examination of the anal area of the HYP-AA-calf showed a necrotizing dermatitis with multifocal acute bleedings and diffuse oedema. In addition, an interstitial pneumonia and urethritis were identified. In the clinical examination of the second case (HYP), a penile and preputial aplasia, an incomplete ventral covering by skin and a bifid scrotum were found. Histopathological examination of the HYP-calf revealed a urethritis with epithelial hyperplasia in the urethra and a follicular hyperplasia of the lymph nodes. The HYP-calf had normally developed testicles. However, there was no active spermatogenesis. The inbreeding coefficient for the HYP-AA-calf was 0.684% and for the HYP-calf 5.273% and both affected calves had one ancestor in common, a Holstein bull used in artificial insemination. In conclusion the congenital anomalies of the two cases might be due to mutations transmitted by the common ancestor.

Keywords: Atresia ani, Congenital anomaly, Hereditary, Hypospadia, Rudimentary external genitalia.

İki Alman Holştayn Buzağıda Atresia Ani, Hypospadia ve Gelişmemiş Dış Genital Organlar Olgusu

Öz: Bu raporda, klinik, patolojik ve sitogenetik muayenelerinin yapıldığı Atreziya ani, hypospadia ve gelişmemiş dış genital organlar ile karakterize bir günlük erkek Alman Holştayn buzağı (HYP-AA) ile Hypospadia ve rudumenter dış genital organlar ile karakterize altı aylık erkek bir Alman Holştayn buzağı (HYP) sunulmuştur. İlk olguda (HYP-AA) anüs oluşmamıştı. İdrar yolu perineuma açılmıştı; bunun sonucu olarak, kasık bölgesinden idrar süzülüyordu. İkinci bulgu olarak, yaklaşık 10 cm'lik bir çapa sahip olan anal bölgede bir vezikül oluşumu vardı. HYP-AA-nın anal bölgesinde yapılan histopatolojik incelemede multifokal akut kanama ve yaygın ödemli nekrotizan dermatit odaklar görüldü. Klinik olarak, interstisyel pnömoni ve üretritis tespit edilmiştir. İkinci olguda ise (HYP), bir penil ve prepusyal aplazi, ventral tarafı deri tarafından kaplanmamış bifid skrotum olgusu görüldü. HYP-nin histopatolojik incelemesinde üretra epitelinde hiperplazi ve lenf nodularında foliküler hiperplazi ve üretritis gözlemlendi. HYP-nin testisi normal gelişmiş, ancak aktif spermatogenez görülmedi. HYP-AA için akrabalık katsayısı % 0.684 ve HYP-için % 5.273 ve her iki buzağının babaları ortakdı. Sonuç olarak iki olguda da meydana gelen konjenital anomaliler ortak boğa tarafından iletilen mutasyonlara bağlı olabilir.

Anahtar Kelimeler: Atreziya ani, Hypospadia, Kalıtsal, Konjenital anomaly, Rudumenter dış genital organlar.

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INTRODUCTION

Congenital defects are structural or/and functional abnormalities that are present at birth. The frequency of congenital defects in cattle was estimated to be at 0.25% (1). Rectal anomalies have been reported in cattle as the most frequent inborn anomalies of the gastrointestinal system (2). Atresia of the intestine can be localized in the ileum, colon, rectum, or anus (3-5). Atresia ani is characterized by perineal bulging and absence of an anal opening. This anomaly is a common birth defect in cattle and is often accompanied by other congenital defects of the digestive, urogenital tract (6-9) or other organs (4,5). Based on varying degrees of dysgenesis or agenesis of the rectum and anus, atresia ani in cattle can be categorized into four types (9). Type 1 involves the development of a relatively normal rectum and a patent but stenotic anus. Type 2, or imperforate anus, is characterized as a rectum that ends in a blind pouch without development of an anus. In most cases the rectum is properly formed but the anus is covered by a thin membrane of skin. Type 3 is characterized by formation of a blind pouch in the proximal rectum and absence of an anus. Type 4 is described by the formation of a blind pouch in the proximal rectum and a normally developed anus. Intestinal atresia has been reported for various cattle breeds such as Holstein, Ayrshire, Charolais, Swedish Highland, Jersey, Maine Anjou, and Shorthorn breeds (3,7,10-13). The pattern of inheritance in cattle is still unclear (3,7). Congenital anomalies of the urinary system are rarely observed in cattle even, so a wide variety of malformations could be encountered (1). Hypospadia is an imperfect closure of the external male urethra (14) with dribbling of urine in the inguinal region. This abnormality can be observed as a glandular, penile, scrotal, and perineal imperfect closure of the urethra (14,15). Hypospadia is the second most common congenital abnormality of the urogenital system after cryptorchism in humans (16) but appears rarely in animals (15,17). Further defects associated with hypospadia are cryptorchism, atresia ani, bifid scrotum, penile aplasia and omphalophlebitis (Table 1). Herzog (14) suggested multifactorial genetic, endocrinological and environmental factors to cause this malformation. In a study on rats, Uda et al. (18) assumed defects in androgen metabolism and/or androgen receptors which may lead to this congenital deformity.

Table 1: Review of single cases of *hypospadia* in cattle.
Tablo 1. Sığırlarda tekli hypospadia vakalarının literatür taraması.

Reference	Breed/sex	Age	Type	Urogenital anomaly	Other anomalies
Alam et al. 2005	Korean/m	2-3 weeks	scrotal	*penile aplasia*, unilateral *cryptorchism*	none
	Korean/m	2-3 weeks	perineal	*penile aplasia*, unilateral *cryptorchism*	none
	Korean/m	2-3 weeks	perineal	*penile aplasia*, unilateral *cryptorchism*	none
Torres et al. 2013	Nelore/m	1 days	scrotal, anal, perineal	*bifid scrotum*	*atresia ani* and *recti*, *omphalophlebitis*
Pamuk et al. 2010	Simmental/m	1 days	perineal	*penile* and *preputial aplasia*, rudimentary left kidney and left urethral anomaly	none
Abd-El Hady and El-Din 2014	crossbreed/f	5 months	penile	*penile* and *preputial hypoplasia*	none
Kumi-Diaka and Osori 1979	Bunaji/m	1 day	scrotal	*bifid scrotum, preputial aplasia*	*atresia ani*
	Bunaji/m	1 day			*atresia ani*, imperforated left ear

The objective of the present report was to describe a case of hypospadia in a six months old male German Holstein calf and a case of atresia ani and hypospadia in a one-day old male German Holstein calf associated with rudimentary external genitalia using clinical, histological, pathological, and cytogenetic examinations as well as pedigree analyses.

CASE REPORT

Case History

A case of atresia ani (HYP-AA) and a case of hypospadia (HYP) were notified in Holstein calves on dairy farms. The male HYP-AA affected calf was born after full term gestation in July 2009 (Figure 1A). At the age of one day, the HYP-AA-affected calf and at the age of six months the HYP-affected calf were transferred to the Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover (Figure 2A). The dam of the HYP-affected calf gave birth to one healthy calf before. Both farms were free of bovine viral diarrhoea (BVD) virus and bovine herpes virus type 1 (BHV1).

Clinical Signs

The present HYP-AA-case had no anal opening (Figure 1A). There was a long subcutaneous skin groove extending from the perineal region towards the penis and rudimentary external genitalia (Figure 1B, 1C). The behaviour of the affected calf was very calm. The rectal temperature was markedly lowered (35.4°C). Furthermore, bradycardia (76/min) and hypopnea (15/min) were observed. The calf died a few minutes after clinical examination.

The HYP-case skinned an opening of the urethra in the perineal region below the anus. The animal was at good health. The animal showed an internal body temperature of 38.5°C, a heart rate of 80 beats per minute and a normal respiration rate of 25 per minute. After six month the calf was slaughtered at a weight of 150 kg.

Figure 1. A: Holstein calf affected by *atresia ani* and *hypospadia*(HYP-AA). **B:** Type 2 *atresia ani* with a missing anal orifice. **C:** *Hypospadia* (a), *penile* and *preputial aplasia* (b). **D:** Preparation of the testicles, urinary tract and bladder.

Şekil 1. A: Atrezia ani ve Hypospadidan etkilenmiş Holştayn buzağı (HYP-AA). **B:** Anal deliği oluşmamış Tip 2 atreziya ani. **C:** Hypospadia (a), penil ve prepusyal aplazi (b). **D:** Testisler, idrar yolları ve mesane görünümü.

Figure 2. A: Holstein calf affected by *hypospadia* (HYP). **B:** Ventral area of the *urethra* of the present case with the perineal area (1), scrotal area (2) and penile area (3). **C:** *Penile* and *preputial aplasia* (1).
Şekil 2. **A:** Hypospadiadan etkilenmiş Holştayn buzağı (HYP). **B:** Olgunun üretrasının ventral alanının perineal (1), skrotal (2) ve penil (3) bölgesi. **C:** Penil ve prepusyal aplazi (1).

Necropsy Findings

The anal subcutaneous skin of the HYP-AA-affected calf showed a severe purulent necrotizing, focal dermatitis and a diffuse oedema (Figure 1B). The penis was located 3 cm cranial to the genital region (Figure 1C). In this area there were 3 ducts. The medium duct ended at a diameter of about 3 cm and was in the digestive diverticulum, which showed multiple, subepithelial, petechial haemorrhages. The second duct ended anterior to the anal region in a skin opening, which was approximately 10 cm long and presented as a diffuse drain channel in the skin. At the end of the drain channel skin flaps of approximately 3 cm were in a rudimentary trained prepuce. The third duct was surrounded by connective tissue and could be traced to the preputial area. The atresia was approximately 10 cm

cranial to the anus and showed an engorgement of meconium in the descending colon and rectum. The cecum showed a clockwise torsion by 180° with prominent haemorrhagic infarction of the mucosa (Figure 3A). In the lungs of the present case a low acute diffuse congestion hyperaemia was observed. Furthermore, a little to medium grade pneumonia was detected (Figure 3B).

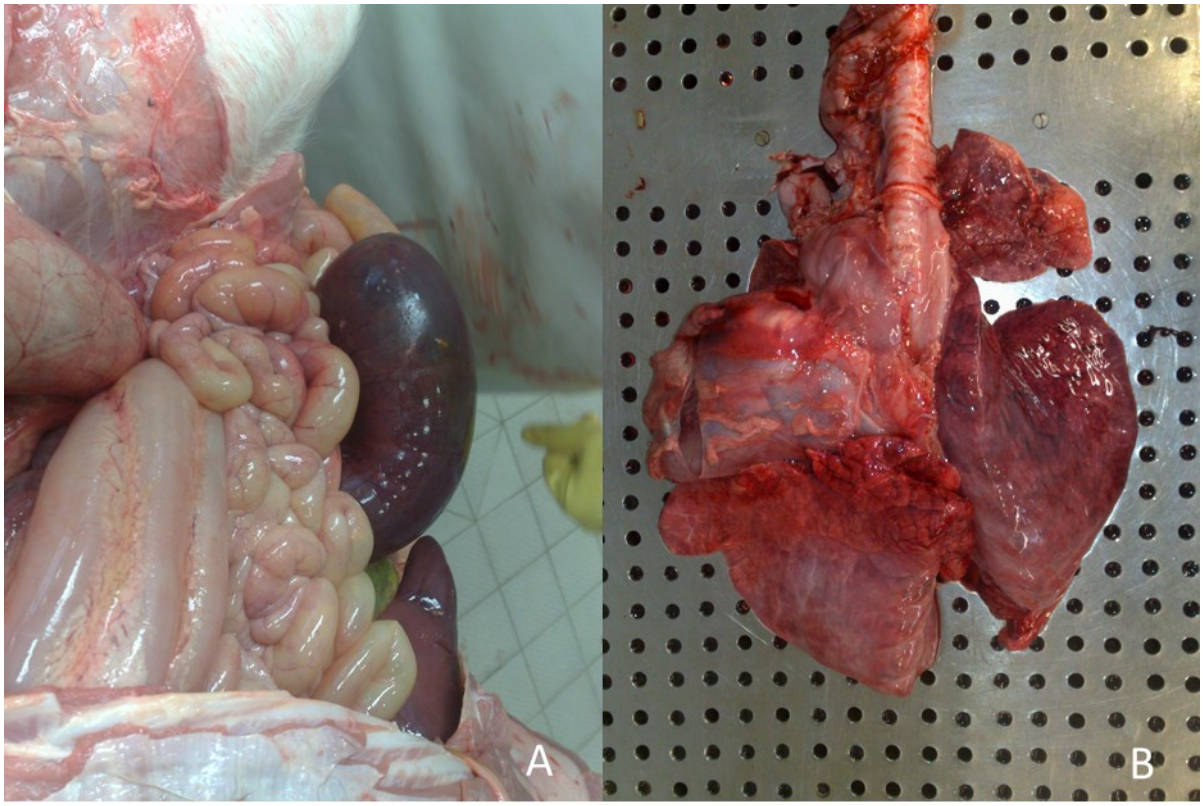

Figure 3. Necropsy of the Holstein calf affected by *atresia ani* and *hypospadia* (HYP-AA). **A:** Clockwise torsion of the *cecum* by 180° with prominent haemorrhagic infarction of the mucosa. **B:** Congestion hyperaemia and alveolar and interstitial pneumonia and oedema in the lungs of the present case.
Şekil 3. Atrezia ani ve hypospadidan etkilenmiş Holştayn buzağının nekropsisi (HYP-AA). **A:** Mukozanın belirgin hemorajik enfarktüsü ile karakterize *cecum*'un saat yönünde 180 ° dönmesi. **B:** Mevcut olgunun akciğerlerinde hiperemik bir görüntü ile alveolar ve interstisyel pnömoni ve ödem oluşumu.

Histopathological Findings

After necropsy of both calves, tissue samples were fixed in 10% buffered formalin, dehydrated in alcohol series, and embedded in paraffin paraplast mixture for histopathological analysis. The paraffin block sections (2-3µm) were prepared using a rotary microtome with integrated Super frost/Plus slides and stained mechanically in a colour machine (Leica ST 4040, Nussloch) with Hematoxylin-Eosin (HE). The tissue of the anal area showed a severe, necrotizing dermatitis with multifocal acute bleeding and a diffuse oedema. In the umbilical artery a severe, fibrino-purulent periarterial inflammation was observed. The lungs showed a medium-grade alveolar oedema, a low-grade interstitial oedema and a mild, multifocal, interstitial pneumonia. In the urethral diverticulum the transitional epithelium of the lamina propria was oedematous. The urethra showed a purulent urethritis with a severe, ulcerative dermatitis and development of granulation tissue. The urogenital tract of the HYP-affected calf endeavoured a histopathological examination. The closed part of the urethra showed a moderate multifocal lymphohistiocytic urethritis. At the transition of the closed to the open part of the urethra cutaneous mucosa was observed. On the open part of the urethra, a moderate epithelial

hyperplasia was detected. The testicles were normally developed. However, there was no active spermatogenesis. The regional lymph nodes showed a low-grade follicular hyperplasia.

Virological Findings

The blood samples were tested for bovine viral diarrhea (BVD) virus antigen (Ag) by ELISA (cooker check BVD Ag/Serum Plus, IDEX Laboratories) and for BVD virus antibody (Ab) (cooker check BVDV Ak, IDEX Laboratories) at the Institute for Virology. The virological results of the present cases were negative for bovine herpesvirus 1 (BHV-1), bovine viral diarrhea (BVD) virus, bluetongue virus 8 and parainfluenza virus 3.

Pedigree Analysis

The six prior generation pedigree data of the calves were analysed using the program Opti-Mate, version 3.81 (19). The dam of the HYP-AA-calf gave birth to eight healthy calves before. There were no cases of atresia ani or hypospadia before in this herd. All heifers and cows had been bred by artificial insemination (AI). The sire of the HYP-AA-calf was an Austrian Red Holstein AI-bull. The inbreeding coefficient for the AA-calf was 0.684% due to a common ancestor on the maternal and paternal side. The dam of the HYP-calf gave birth to one healthy calf before. The farm had no cases of hypospadia or other anomalies before. The inbreeding coefficient for the HYP-calf was 5.273% due to a common ancestor on the maternal and paternal side. Using pedigree records, we traced the on the paternal side two affected calves to one common ancestor, a common Holstein AI-bull. The sires of both affected calves had this Holstein bull as common paternal grandsire (Figure 4).

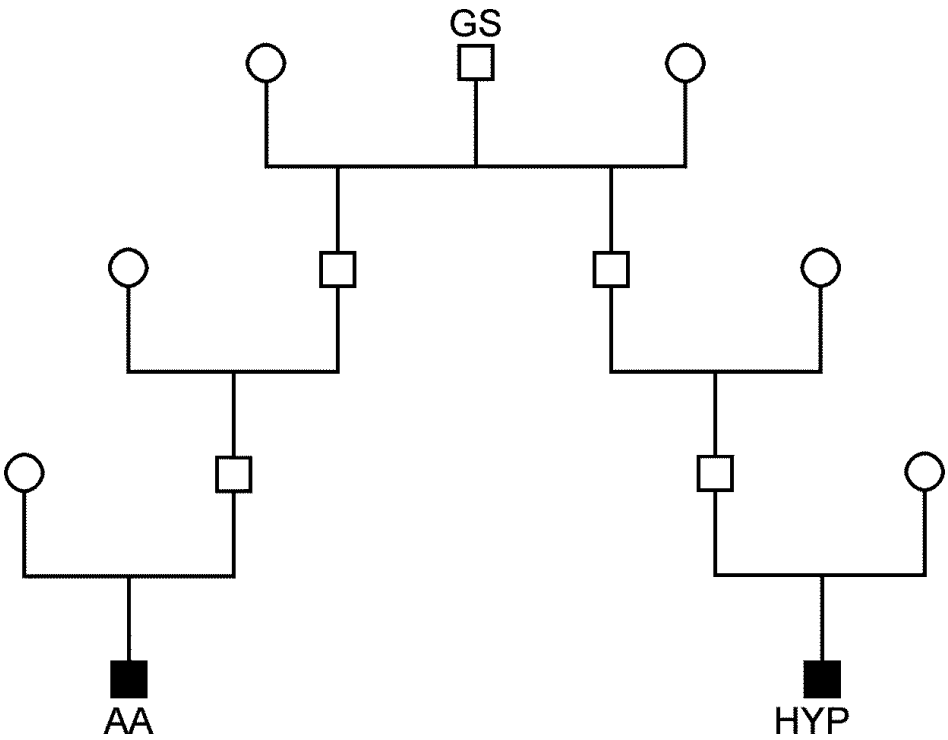

Figure 4. Pedigree of the calves affected by atresia ani and hypospadia(AA) and affected by hypospadia (HYP). GS=Grandsire
Şekil 4. Atreziya ani ve hypospadia (AA) 'dan etkilenen ve hipospadiya (HYP)' dan etkilenen buzağıların pedigrisi. GS = Büyükata

Chromosomal findings

Cytogenetic analysis was performed by standard methods from blood lymphocytes. Metaphase spreads were prepared from fibroblast cultures, G banded, and Giemsa stained. The arrangement of the chromosomes in the karyotype corresponds to the international nomenclature (International System for Chromosome Nomenclature of Domestic Bovids, 2000). The cytogenetic results of both calves revealed no discernible difference in the number of chromosomes. The calves showed normal karyotypes of male cattle with 2n= 60,XY.

DISCUSSION and CONCLUSION

The inborn anomalies the two calves had in common were hypospadia, a rudimentary penis and a rudimentary preputium as well. Both calves could be traced back to a common ancestor, a Holstein bull used in artificial insemination (AI).

The hypospadia of both affected calves could be classified as penile, scrotal, and perineal form due to its extension. Hypospadia appears most often in dogs, goats, and sheep. In contrast, in cattle hypospadia occurs only infrequently (0.46%) (20). In men, mutations in the genes HOX, FGF, WT1, MAMLD1, SRD5A2 and AR seem to be associated with hypospadia (21). In cattle, a recessive mode of inheritance had been assumed (14).

The common Holstein AI-bull used in artificial breeding may be associated with the development of the condition of hypospadia. The formation of urogenital anomalies was reported to be caused by chromosomal anomalies (22). In contrast to the results of Rousseaux and Ribble (22), the calves of the present report showed normal male karyotypes of 2n= 60,XY.

Congenital anomalies have been rarely described after in vitro-production (IVP) of embryos in cattle. In a study on more than 2000 calves, King et al. (23) found two calves with kidney failure, hydrocephalus, abnormal hearts, and cerebellar aplasia. In the HYP-AA-affected calf from IVP, atresia ani with hypospadia and rudimentary genital organs were diagnosed. Other authors described the presence of atresia ani and an underdeveloped preputium and scrotum in calves born after cloning (24,25).

Based on the scheme according to Loynachan et al. (9), type 2 atresia ani was diagnosed in the HYP-AA-case. Clinical signs of atresia ani seen in the HYP-AA-affected calf including anorexia, abdominal distension, decreased suckle reflex (3,26) and no passing of meconium or faeces were consistent with previous reports. The most frequently encountered urogenital abnormalities in calves are hypospadia, hermaphroditism and penile agenesis (7-9). Hypospadia is a congenital defect of the urethra that involves an abnormally positioned urinary meatus. Dependent on the anatomical localization of the urethral opening this condition is classified as anal, perineal, scrotal, or penile hypospadia. In men, hypospadia is usually of the penile type, whereas in cattle the perineal and scrotal hypospadia occur most frequently.

The reports on hypospadia or atresia ani in cattle are most often dealing surgical treatment. This is the first study in cattle on two inbred cases with hypospadia and related through a common ancestor used in AI. Therefore, hereditary factors may be involved in hypospadia.

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