

Long-Term Pedigree Analysis: An Effective Tool for Managing Congenital Malformations in Cattle

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Simple Summary: Controlling congenital defects is an important aspect of breeding for genetic health; however, whether malformations are caused by genetic or nongenetic factors may not be clear. The pedigrees of calves affected by congenital defects aplasia genitalis, atresia ani et recti and hernia cerebialis were analyzed. The calves affected by aplasia genitalis had common ancestors in all cases, and the results indicate hereditary causation with recessive inheritance. Additionally, in atresia ani et recti, some pedigrees of affected calves support hereditary causation. In contrast, the analysis of hernia cerebialis cases did not confirm the genetic background. The pedigree analysis of congenital defects could help in managing genetic health, although the final goal in terms of inherited defects must be the description of causal genes and mutations.

Abstract: This study aims to analyze the pedigrees of calves affected by one of three congenital defects: aplasia genitalis, atresia ani et recti and hernia cereбрalis. The defects were diagnosed by veterinary surgeons in the Czech surveillance program for bovine genetic disorders. The sires fathering affected calves were born in the Czech Republic or imported from 1986-2001. The cases occurred on farms across the Czech Republic. The pedigree of each case was examined for common maternal and paternal ancestors (inbreeding loops) and for ancestors shared by other cases of the defect. The 13 calves affected by aplasia genitalis had common ancestors in all cases. The results indicate hereditary causation with recessive inheritance. Additionally, in atresia ani et recti, some of the pedigrees of 25 affected calves support hereditary causation, and repeating ancestors were found for 11 calves. In contrast, our analysis of 11 hernia cereбрalis cases did not confirm the genetic background. We demonstrated that the pedigree analysis of congenital defects could help in controlling genetic health, although the final goal in terms of inherited defects is the description of causal genes and mutations. Measures to control sires that father affected calves should be appropriate under the current knowledge, which include culling or prudent use of breeding with the monitoring of descendants.

Keywords: genetic health; surveillance program; aplasia genitalis; atresia ani et recti; hernia cereбрalis

1. Introduction

The ultimate goal of breeding is to produce animals with a genetic background of high production and sound health. Genetic health must be ensured at all levels, meaning that the genome should be free of mutations of individual genes and free of chromosomal abnormalities and aneuploidies, and the health the animals, including the animal's fertility, which is controlled by multiple minor genes, should be improved continuously as well. The control of congenital malformations plays an important role in the breeding process. In humans, approximately 50% of congenital anomalies cannot be linked to a specific cause. However, known cases include single gene defects, chromosomal disorders, multifactorial inheritance, environmental teratogens and micronutrient deficiencies. Genetic cases can be traced to inherited genes or mutations [1].

The etiology of congenital defects in farm animals is often unclear because many teratogens, including infectious agents, may be involved, and whether a defect is caused by acquired or inherited defects can be uncertain. Some congenital defects occur only rarely; therefore, thorough analyses of these defects is difficult. In some cases, the genetic background can be uncertain and whether the defect is caused by monogenic or polygenic factors or dominant or recessive inheritance can be dubious as well [2].

The occurrence of congenital malformations in livestock production, especially when caused by gene variants passed down by parents, hinders genetic progress. To study the genetic bases of these diseases and find strategies to eradicate them in livestock populations is an issue that breeders must overcome to avoid economic losses [3,4]. Deleterious genetic conditions need to be recognized because they limit the productivity of animals expressing the trait. Affected animals should not be allowed to reproduce to benefit the production system [5].

Aplasia genitalis is the lack of development of female genitalia. The most extreme abnormality of Müllerian ducts is congenital absence of the uterus and vagina (CAUV), and the

etiology is still unclear [6]. Congenital atresia of the vagina in the region of the hymen sometimes occurs in heifers.

Phenotypic similarity to the mentioned defects shows white heifer disease or Müllerian ducts hypoplastic 1 (MDHO1). The affected females are sterile, the etiology is hereditary, and defects occur mainly in Belgian blue and Shorthorn white heifers [7]. The differentiation and development of genitals is suppressed, but the ovarian function and periodicity are maintained [8,9]. The defect is caused by the Roan gene in BTA5. Similarities in the causation of aplasia genitalis in other breeds have not been clarified.

Studies based on targeted mutagenesis in mice have identified genes participating in the development of Müllerian ducts [10]. Genital hypoplasia is an inherited defect without published evidence for single-locus inheritance (Online Mendelian Inheritance in Animals OMIA number 000406-9913) [11].

Additional defects with different etiologies are hypoplasia bisexualis and freemartinism. The signs are larger clitoris, small blind vagina, hypoplasia or aplasia of the uterus. The syndrome is triggered by placental anastomoses between fetal membranes of heterosexual twins, and androgenic hormones suppress normal development of female gonads [12-14]. However, in a comprehensive publication on the Genetics of Cattle, freemartinism is listed as an inherited disorder for which there is insufficient published evidence for single-locus inheritance (OMIA number 000393-9913) [11,15].

Intestinal atresias are inborn defects of mammals, including humans. Atretics can occur in the gut from the duodenum to the anus [16,17]. Thus far, the causation has not been definitively elucidated. Although genetic and nongenetic reasons remain possible, the prevailing assumption is that the defect is hereditary and caused by an autosomal recessive allele [11,18].

In cattle, atresia of the large intestine and anus is the most common form [16]. The latter is easily diagnosed by breeders a few days after delivery, and surgical treatment has a good prognosis. The frequency in male and female calves is reported to be even [19,20].

Hernia cerebialis or encephalocele is the herniation of the brain into a sac covered with a membrane. The defect likely does not occur because of faulty closing of the neural tube; rather, it is likely caused by later damage to superficial ectodermal structures of the already closed neural tube [21]. Such congenital defects of the skull also occur in other mammals, including horses, pigs, rabbits and humans [22-25].

Cranial meningocele (containing only the meninx) has been well documented in the Ayrshire calf, and cranioschisis with meningocele has been well documented in the Shorthorn calf and is associated with additional anomalies, including tibial hemimelia and abdominal hernia. The Shorthorn calf is a member of a group of related calves that present tibial hemimelia and other defects [25,26].

In humans, the etiology of these defects seems to be multifactorial, polygenic, and epigenetic under the influence of external factors [27]. Namely, occipital encephalocele usually occurs with a monogenic hereditary syndrome, and some causal genes have already been identified. The defect can also be a consequence of trisomy 13 or 18 [28].

This study aims to perform and analyze the pedigree of calves in the Czech Republic affected by one of three congenital defects: aplasia genitalis (AG), atresia ani et recti (AAR) and hernia cerebialis (HC).

2. Material and Methods

Cases of AG, AAR and HC, among other congenital disorders, were diagnosed and reported by veterinary surgeons to the mandatory Czech surveillance program for bovine genetic disorders. No experiments were performed on living animals. Field veterinarians noted the diagnosis of the affected calves and identified the sires and dams. The study was limited to calves fathered by sires born between January 1986 and December 2001. The sires were born in the Czech Republic or included in the herd book due to semen import [2]. The study period is based on the nonmandatory reporting of congenital disorders after 2001. In total, 6047 sires were newly recorded into pedigree books in the period from 1986-2001, and 2740 were Holsteins and 3307 were Czech Simmentals. Seventy-eight percent of Holstein sires and 15% of Simmental sires were imported, mainly as frozen sperm. Of the recorded sires, 474 (7.8%) fathered offspring with different congenital disorders. The latest data are not available because the supervising program has ended. The cases occurred on farms throughout the Czech Republic.

There were 13 reported cases of aplasia genitalis in female calves. In 12 cases, both parents were known, and in 1 case, the mother could not be traced back. Twenty-five calves presented atresia ani et recti. In 21 cases, the sex was not recorded, including two affected males and females. In 21 calves, both parents were known, and in 4 calves, the mother was not reported. For hernia cerebrealis, 11 cases were reported. In 9 cases, both parents were known, and in 2 cases, the mother was not reported. The sex of affected calves was not reported.

The pedigree of each case was examined for common maternal and paternal ancestors (inbreeding loops) and for ancestors shared by other cases. The total offspring for the sires of affected calves was found in the documented breeding materials.

Wright's inbreeding coefficient (F_x) was calculated when a common maternal and paternal ancestor was found by the following equation:

$$F_x = \sum 0.5^{n_1 + n_2 + 1} (1 + F_a)$$

where n_1 and n_2 are the number of generations between the affected calf and the common ancestor in the paternal and maternal parts of the pedigree, respectively. If the common ancestor was inbred, then the coefficient of inbreeding was (F_a).

3. Results and Discussion

The results of the analysis of the incidence of aplasia genitalis are shown in Figures 1-3. Of the thirteen females affected by aplasia genitalis, three were from heterosexual twins, i.e., the causation was freemartinism and nongenetic. These cases were fathered by ten sires, i.e., two percent of 474 sires with affected offspring. The pedigrees consisted of four to eight generations, and unfortunately, they were not complete in all branches, which was similar to the other two congenital malformations studied.

Considering the ten affected calves without freemartinism as a cause, common ancestors were found in all cases. In Figure 1, sire I/2 fathered two affected daughters from different mothers. Figure 2 shows a slightly more complicated pedigree. Sire III/2 fathered three calves with AG, and calves IV/3,4 were from heterosexual twins and thus probably affected by freemartinism. The semibrother III/1 also fathered one calf with AG. The affected calves IV/5,6 had common ancestor I/1. Sire II/4 was the father of one affected calf III/6 and the grandfather of IV/5. Moreover, calf IV/6 was a product of close inbreeding. Additionally, the affected calves in Figure 3 had common ancestors. For the breed of fathers, seven Holsteins prevailed in three crosses of Czech Simmental with Holsteins and Ayrshire. Their inbreeding coefficient was 3.1% on average, with a maximum of 8.8%, and the others had a coefficient of less than 4.9%.

Low inbreeding in the population is laudable because the degree of inbreeding significantly influences production and reproductive parameters [29].

Such results indicate a hereditary background of aplasia genitalis. Furthermore, in all ten pedigrees of affected nonfreemartin calves with common ancestors, both male and female, there was no pedigree without a shared ancestor with another pedigree. However, due to the structure of cattle breeding, common ancestors and thus inbreeding loops would probably have been found by chance and therefore would not provide clear evidence for inheritance. Studies of multiple closely related cases by molecular techniques, such as SNP-based association mapping, would probably be most helpful in demonstrating a genetic etiology [30]. To ensure accurate diagnosis, a uniform collection of cases for molecular examination is needed. Previously described genes with roles in sexual development should be a focus [10]. Moreover, different mutations may be associated with an AG phenotype.

The analysis hints at the recessive inheritance of AG; thus, a count of respective genes should be established. Dominant inheritance is not involved when sires that father daughters with defects also sire many healthy daughters. Moreover, the X-linkage is questionable. The infectious etiology seems to be implausible, and other nongenetic factors are unlikely for cases reported as isolated events spread in different regions.

The nine sires with calves affected by aplasia genitalis (not counting freemartinism) have fathered over fifty thousand descendants of both sexes as well as nine sires. This information is a cause for concern with respect to genetic health in the Czech cattle population, which numbers 360 thousand dairy cows [31]. Moreover, two sires fathering AG were also found in pedigrees of aborted or stillborn calves, and this defect was characterized at the DNA level in BTA18 [11]. Similarly, three sires in pedigrees of AG calves that were slightly more distant from the affected descendants were also found in pedigrees of stillborn calves and calves with schistosoma reflexus.

The 25 calves with atresia ani et recti were fathered by 23 sires, with one sire having three affected descendants and the others sire having one affected descendant. Sixteen sires were Holsteins, one was Simmental, and six were crosses of Simmental, Holstein, and Ayrshire. The F_x of the sires was of 2.7% on average.

The pedigrees of some affected calves support the hereditary causation of atresia. Repeating ancestors to the 5th generation was found for almost half of the calves, i.e., in 11 cases (Figures 4-7).

Figure 4 shows an example of close inbreeding. The affected calf IV/1 had a common great grandfather - ancestor I/1 in the maternal and paternal branches of the pedigree. In Figure 5, defective calves III/1 and 2 had a common grandfather I/1. A similar case is shown in Figure 6, with calf IV/1 presenting an extra generation.

A more interesting case of the pedigree of six calves affected by AAR is given in Figure 7. Forefather I/2 had five affected descendants, V/2-6. His grandson IV/3 fathered three defective calves from three cows. Calf V/4 was a product of close inbreeding, and sire I/2 was an ancestor in both the sire and dam parts of the pedigree. Additionally, calf V/5 was a product of inbreeding, with sire II/3 a grandfather and great grandfather in the dam branch of the pedigree.

In other affected calves, such inbreeding was not found. Notably, some sires with affected calves fathered numerous descendants. Therefore, sire III/7 in Figure 7 sired 57,577 calves, of which 10 sires and 19,899 cows were included in the milk recording program. Sire IV/3 had 12,766 descendants, with 14 sires and 4231 cows. In addition, other sires with breeding male descendants were found, one with 15 and the others with 1, 5 and 14 sons. Moreover, other congenital defects were recorded in descendants of sires fathering calves with atresia ani et recti, with abortions observed in the descendants of eight sires, cleft observed in one calf, AAR with polydactyly observed in two calves, and AAR with acaudia observed in one calf. These alarming results are similar to the case of aplasia genitalis.

The etiology can be definitively resolved by identifying causal genes and mutations. Hereditary causation is commonly considered highly likely. Atresia ani is classified as a defect with monogene heredity but no known causal mutation; and atresia coli, atresia ilei and atresia intestine are considered hereditary defects without evidence for monogene heredity [11,15]. Additionally, our cases of atresia ani et recti were recorded in different regions across the Czech Republic.

Compared with the previous two defects, the pedigree analysis of hernia cerebrealis did not considerably demonstrate a hereditary background. Eleven HC cases were evaluated, and five admitted a possibility of genetic etiology (Figures 8-10). The eleven calves with HC were fathered by ten sires, with one presenting two affected descendants (Figure 10) and the remaining calves presenting one affected descendant.

In Figure 8, Simmental mother IV/1 of the affected calf was a product of close inbreeding; however, the F_x of females is not given in the databases. Sire IV/2 was Charolais, his F_x was 0.063, and the number of descendants was 375.

In Figure 9, mothers III/1 and IV/2 of affected calves have common ancestor I/1. In Figure 10, the two affected calves have a common father II/2 (F_x 0.034), with more than 13,000 descendants. The remaining six affected calves did not demonstrate such close repeated ancestors.

In hernia cerebrealis cases, animals rarely repeat within the pedigrees of affected calves in more distant parts of the pedigrees. Furthermore, the pedigrees were not as interconnected as in aplasia genitals or atresia ani et recti. For ten sires of affected calves in the Czech cattle population, they sired over 21,000 descendants, with sire II/2 in Figure 10 siring more than 13,000. As mentioned above, common ancestors and thus inbreeding loops would probably have been found by chance in many pedigrees. The F_x of sires fathering aplasia genitalis was of 3.1%.

Thus, our results do not provide massive support for the genetic background of the studied cases of hernia cerebrealis. In general, congenital defects can arise as developmental disorders without impaired genetic material. However, a few previous results identified hereditary causation of HC in humans and zebrafish [32]. In the general survey Genetics of Cattle, the condition brain hernia is mentioned as genetic without evidence for one-locus inheritance [15]. According to OMIA (number 0457-9913), the defect was listed in cattle as a definite single-locus disorder, although the evidence for such a claim is insufficient because the only evidence is a brief and old report of a single case by Shaw (1938), which also mentioned that a still-born full sister also had the defect [11,33]. Therefore, it is doubtful whether hernia cerebrealis should be listed as a genetic defect in cattle, and the defect should be studied intensively.

Pedigree analyses are often performed in studies of population structure and genetic variability for conservation programs [34]. Applications in studies of congenital defects are not common. However, such work could be beneficial for preliminary research of afflictions without known etiology. The advantage is that pedigree analyses could be performed even if biological samples are not available, thus preventing examination at the gene level; moreover, old cases could be involved when pedigree information is conscientiously documented.

4. Conclusions

The results presented herein support the hereditary causation of aplasia genitalis and atresia ani et recti but did not confirm the genetic background of hernia cerebrealis. The contribution of pedigree analyses to the study of congenital malformations is important, but the final goal must be the identification of causal genes and mutations. In any case, breeding for genetic health requires the systematic recording of congenital defects and strict evaluation of their occurrence in descendants of every sire. Measures against sires fathering affected calves should follow the state of current knowledge. When the causation is hereditary, the sire must be culled. If the

etiology is not obvious, then the sire could be used prudently in breeding but not for the fertilization of elite females, i.e., mothers of sires, and the descendants must be monitored conscientiously. Unfortunately, analyses of congenital defects are not performed systematically in the Czech Republic.

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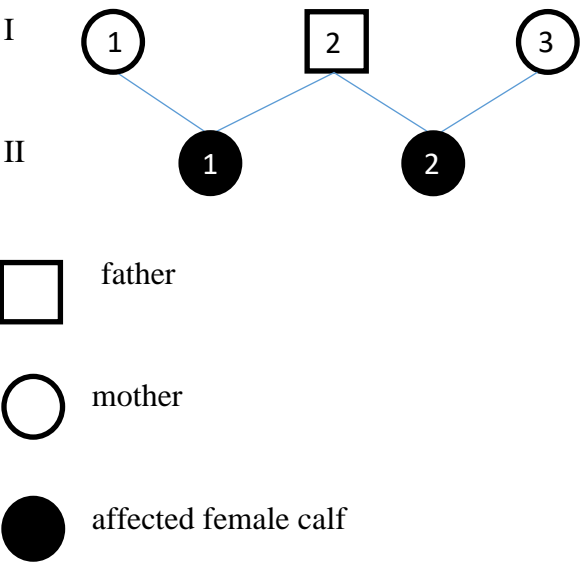
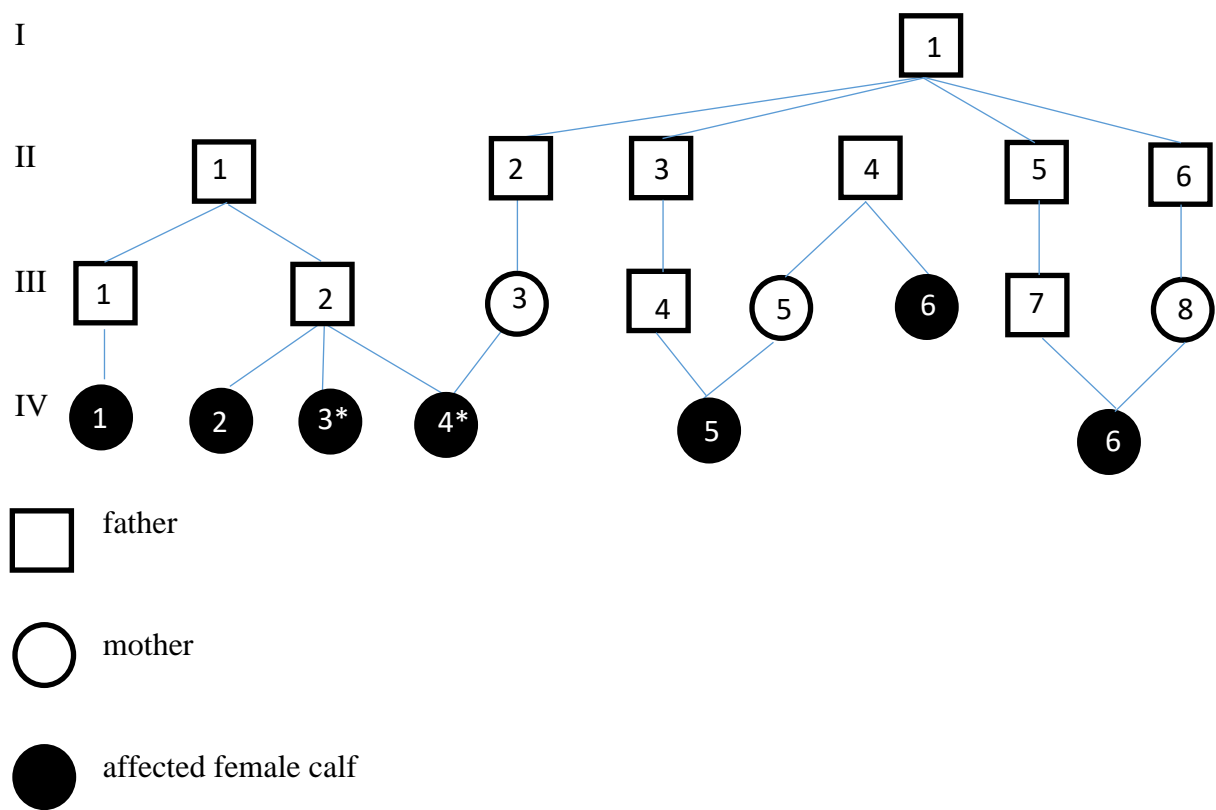


Figure 1. Pedigree of two female calves affected by aplasia genitalis



*Heifer had a twin brother, and the causation is probably freemartinism

Figure 2. Pedigree of six female calves affected by aplasia genitalis

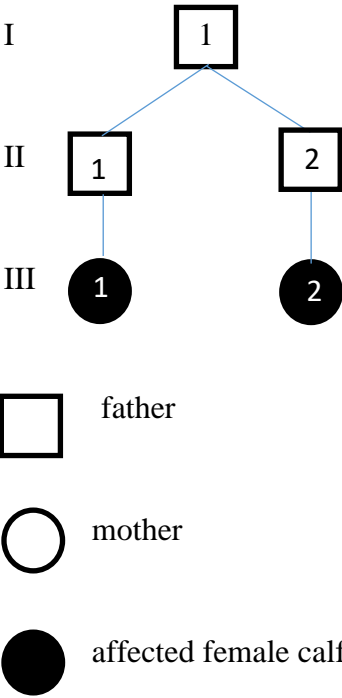


Figure 3. Pedigree of two female calves affected by aplasia genitalis

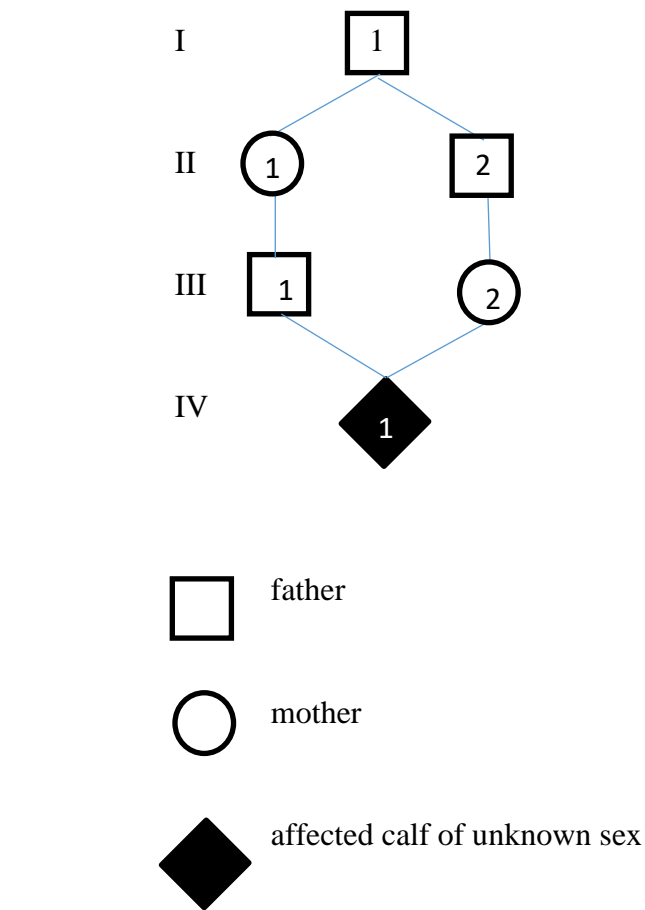


Figure 4. Pedigree of calf affected by atresia ani et recti

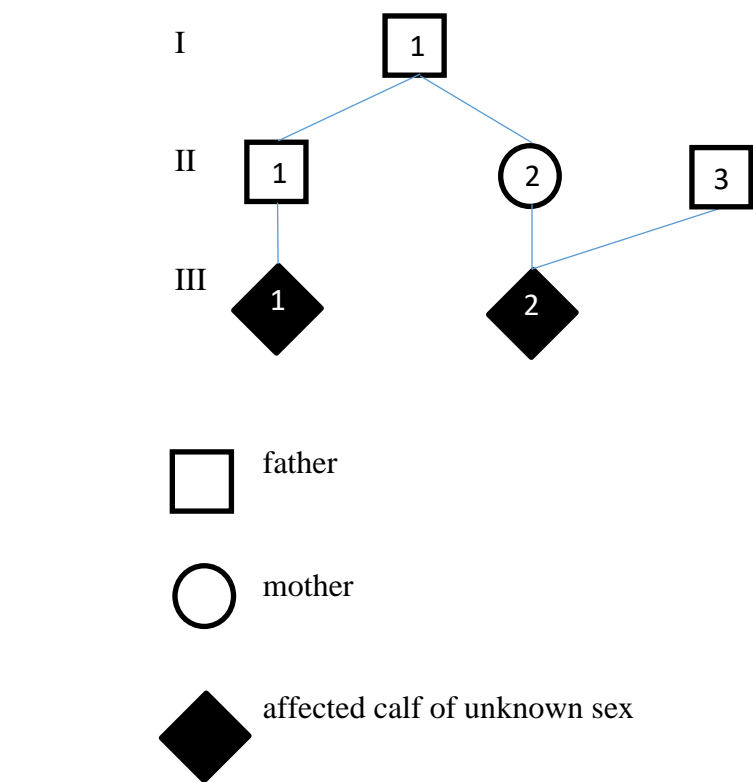


Figure 5. Pedigree of two calves affected by atresia ani et recti

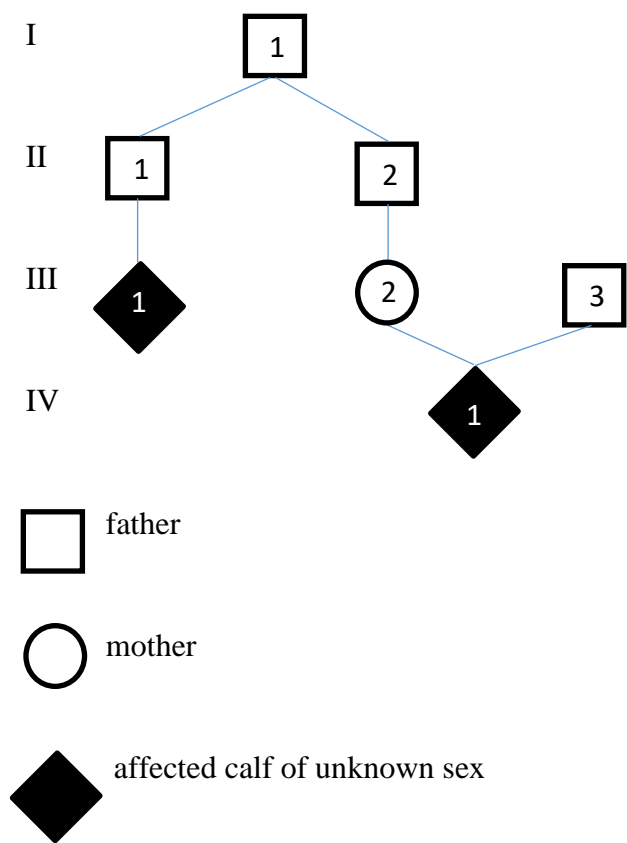


Figure 6. Pedigree of two calves affected by atresia ani et recti

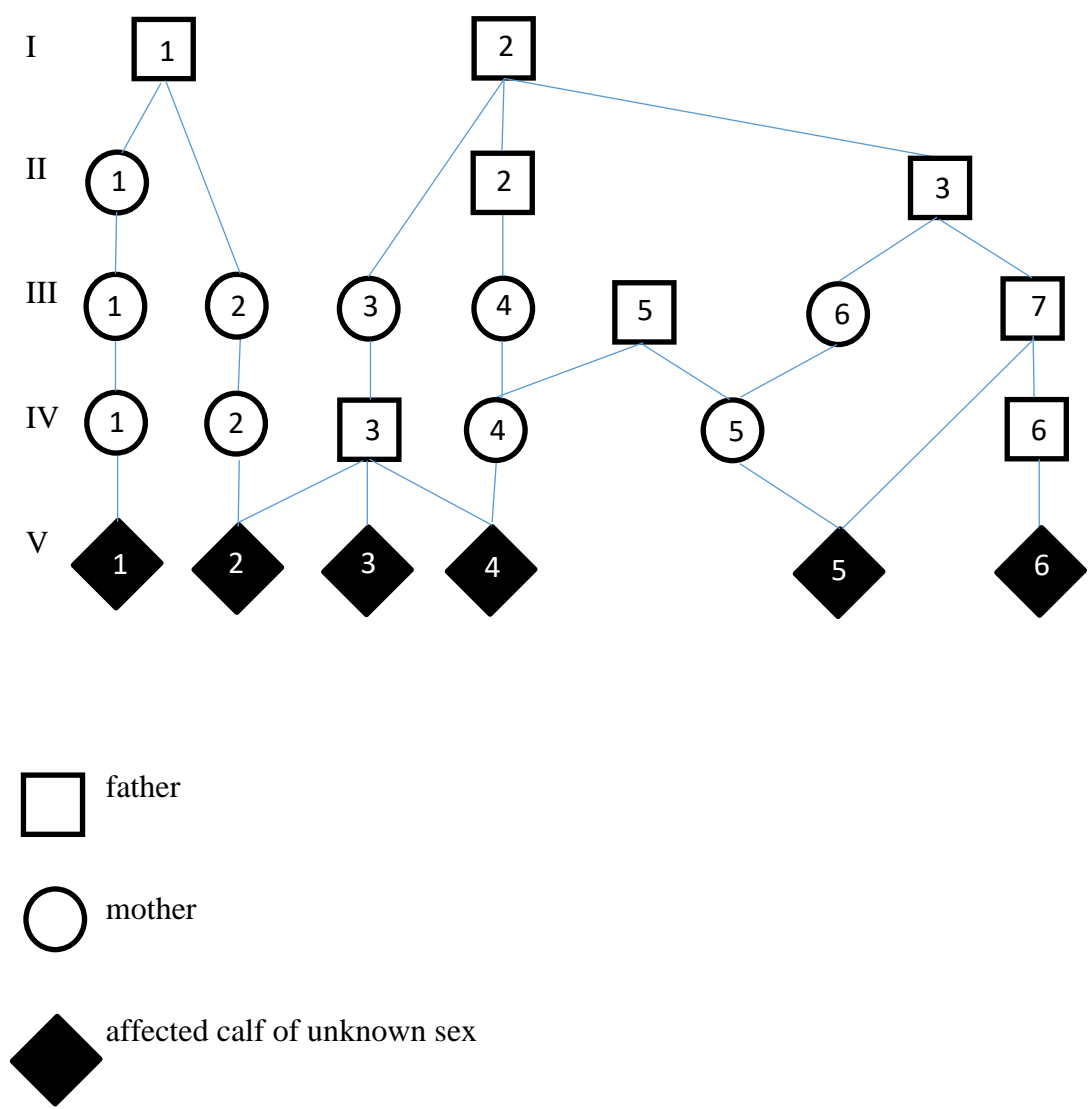


Figure 7. Pedigree of calves affected by atresia ani et recti

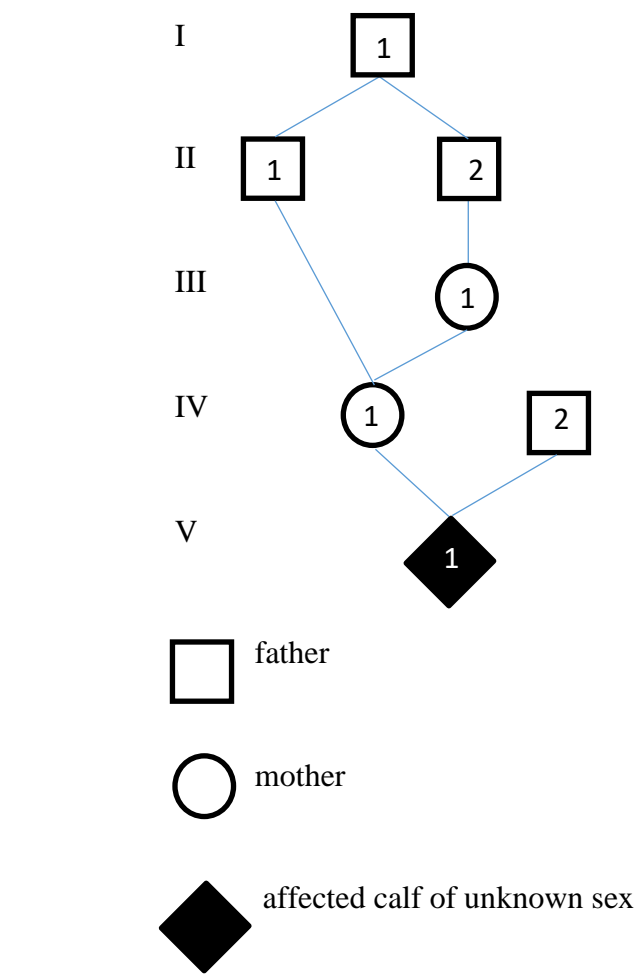
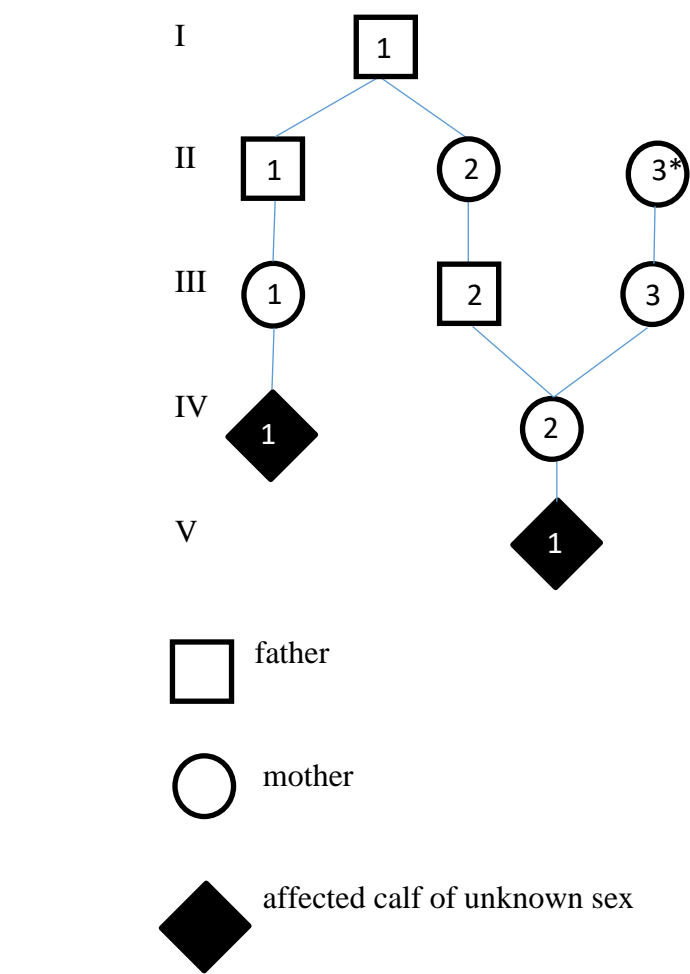
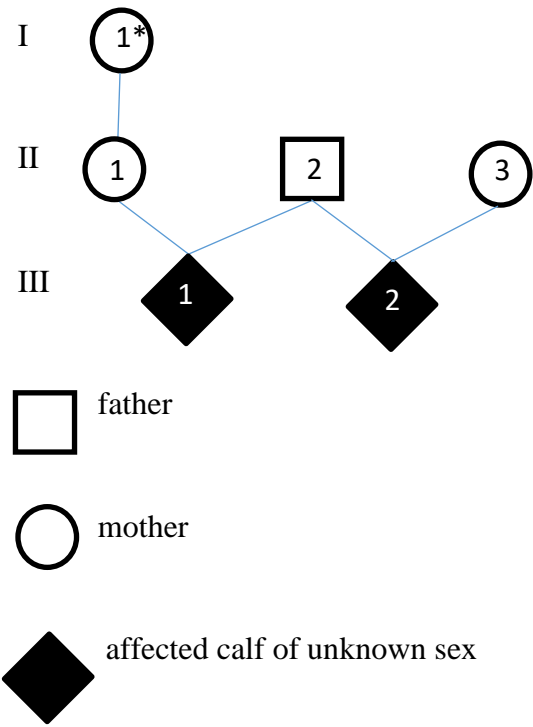


Figure 8. Pedigree of calf affected by hernia cerebrales



*Cow sired another calf with a nonspecified defect

Figure 9. Pedigree of calves affected by hernia cerebrales



*Cow sire another calf with a nonspecified defect

Figure 10. Pedigree of calf affected by hernia cerebrealis