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# Fragile Foal Syndrome: Understanding Its Genetic Basis and Implications for Equine Breeding Practices

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## Abstract

Fragile Foal Syndrome (FFS) is a fatal autosomal recessive inherited disorder initially seen in some warmblood horses, but since has been identified in other breeds such as Thoroughbreds. It is a point mutation in the PLOD1 gene, which is responsible for both collagen production as well as maintaining the integrity of connective tissue. This mutation results in the interference of the collagen synthesis, resulting in deadly physical deformities, such as hyperextensible skin and hypermobile joints, which results in death within hours. FFS was first identified in Warmblood horses, hence the original name, Warmblood Fragile Foal Syndrome, but since has been found to affect a wider variety of breeds, including Thoroughbreds and Haflingers. This recent discovery has raised concerns on the incidence of the disorder and its transmission throughout the various equine populations.

This thesis conducts a comprehensive literature analysis to investigate the genetic underpinnings and clinical manifestations of FFS, as well as its implications for equine breeding, both economically and practically. Similar disorders resulting in collagen dysfunction, such as Ehlers Danlos Syndrome (EDS) in humans and Hereditary Equine Regional Dermal Asthenia (HERDA) in horses are also compared. This highlights a larger genetic and pathophysiological framework that may provide insights for both human and veterinary medicine.

Additionally, this paper discusses new developments in diagnostic techniques such as the creation of a rapid and affordable qPCR assay that makes it easier to identify FFS carriers and may be crucial in reducing the incidence of the disorder through genetic screening. This new technology benefits breeders, allowing them to make responsible breeding choices, and mitigate the risk of producing sick foals. However, difficulties may still arise due to the reluctance of breeders to use genetic testing due to possible implications, affecting their income.

The findings of this thesis highlight the need for more advocacy and genetic knowledge in the equine breeding industry. By promoting awareness of genetic diseases and supporting genetic screening, it is possible to reduce the prevalence of FFS and continue with research to improve equine genetics.

## Absztrakt

A törékeny csikó szindróma (Fragile Foal Syndrome - FFS) autoszómális, recesszíven öröklődő halálos kimenetelű betegség, amelyet kezdetben néhány melegvérű lófajtában észleltek, de azóta újabb fajtákban, például telivérben is kimutatták. A betegséget a PLOD1 gén pontmutációja okozza, amely mind a kollagéntermelődésért, mind a kötőszövet integritásának megőrzéséért felelős. Ez a mutáció a kollagénszintézis megzavarását eredményezi, ami halálos fizikai deformitásokhoz, például vékony, törékeny bőr és nyálkahártyák jellemzik, valamint és hipermobilis ízületekhez vezet, amik nyílt elváltozásoknak vannak kitéve és végül halálhoz vezet. Az FFS-t először melegvérű lovakban azonosították, innen ered az eredeti elnevezés, a Warmblood Fragile Foal Syndrome, de azóta kiderült, hogy a fajták szélesebb körét érinti, beleértve a telivéreket és a haflingiket is. Aggodalomra ad okot a rendellenesség magas előfordulási gyakorisága és újabb fajtákba való átadásának kockázata.

Ez a dolgozat átfogó szakirodalmi elemzést végez az FFS genetikai hátterének és klinikai megnyilvánulásainak, valamint a lótenyésztésre gyakorolt gazdasági és gyakorlati vonatkozásainak vizsgálatára. Hasonló, kollagén működési zavarokat okozó rendellenességeket is bemutat, mint például az Ehlers Danlos-szindrómát (EDS) emberekben és a Herditary Equine Regional Dermal Asthenia (HERDA) lovakban. Ez rávilágít a nagyobb genetikai és kórélettani együttesre, amely betekintést enged mind a humán-, mind az állatgyógyászat számára.

Ezenkívül a dolgozat a diagnosztikai technikák új fejlesztéseit is tárgyalja, például a gyors és megfizethető qPCR-vizsgálat megvalósítását, amely megkönnyíti az FFS-hordozók azonosítását, és kulcsfontosságú lehet a rendellenesség előfordulásának csökkentésében a genetikai szűrés révén. Ez előnyös a tenyésztők számára, lehetővé téve számukra, hogy felelős tenyésztési döntéseket hozzanak, és csökkenti a beteg csikók előfordulásának kockázatát. Nehézségek adódhatnak azonban, ha a tenyésztők a járulékos költségek növekedése miatt vonakodnak a genetikai vizsgálattól.

A disszertáció eredményei rávilágítanak arra, hogy a lótenyésztési ágazatban több érdekérvényesítésre és genetikai tudásra van szükség. A genetikai

betegségekre való figyelem felkeltésével és a genetikai szűrés támogatásával csökkenthető az FFS pervalenciája, és folytatható a ló örökletes terheltségeinek feltárására és kiküszöbölésére irányuló kutatás.

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## Abbreviations

| | |
|---|---|
| FFS | Fragile Foal Syndrome |
| EDS | Ehlers Danlos Syndrome |
| HERDA | Hereditary Equine Regional Dermal Asthenia |
| NFS | Naked Foal Syndrome |
| LH1 | Lysyl Hydroxylase 1 enzyme |
| HYPP | Hyperkaliaemic Periodic Paralysis |
| SCID | Severe Combined Immunodeficiency |
| DAPI-banding | 4’6-diamidino-2-phenylindole |
| FISH | Fluorescence In Situ Hybridization |
| BAC | Bacterial Artificial Chromosome |
| PPIB | Peptidylprolyl isomerase B |
| SDS | Sodium Dodecyl Sulfate |
| EDTA | Ethylenediaminetetraacetic Acid |
| ddNTP | Dideoxynucleotides |
| NGS | Next Generation Sequencing |

# 1 Introduction

Fragile foal syndrome is a monogenic autosomal recessive condition seen in newborn foals. It was formerly known as Warmblood Fragile Foal Syndrome, as initially it was thought to only affect warmbloods, however, upon further research, the genetic mutation responsible is not exclusive to warmbloods, as It has also been detected in other breeds. This fatal disorder results in the point mutation of the PLOD1 gene, which encodes for the enzyme lysyl hydroxylase, which, by hydroxylation, converts lysine to hydroxylysine. These hydroxylysines act as "precursors for cross linking that are responsible for the tensile strength, mechanical stability of collagen fibrils and are involve in the formation of fibres" [1].

Ehlers Danlos Syndrome is an umbrella of disorders that occur in humans due to the mutation of the PLOD1 gene. It was named in honour of Edvard Ehlers and Henri Alexandre Danlos, two physicians who, in the early 1900s, were the first to describe this particular condition [2]. Unlike in FFS, humans can live a normal lifespan, however, there is an increased risk of extreme complications that can occur, for example- arterial rupture, breathing difficulties, and cardiac failure [3]. There are over 30 mutations of the PLOD1 gene that can occur, resulting in kyphoscoliotic type Ehlers Danlos Syndrome. Both FFS and EDS share similar abnormalities with the connective tissue due to the PLOD1 gene mutation, with clinical signs in humans such as hypotonia, hyper mobility of joints, hyperextensible skin and kyphoscoliosis [3].

Foals that are homozygous positive exhibit severe malformations from birth such as thin, frail, hyper-extendible and easily torn skin. The weakened connective tissue in the joints can result in them being hyper mobile, often to a pathological degree, leading to dislocations and even mobility issues. Unfortunately, there is no cure for FFS and so foals either die within hours or are humanely euthanised to avoid the debilitating suffering that they would inevitably endure [4].

Although, as mentioned, there is no cure for FFS, there are, however, genetic tests that are available to identify carriers. There are no clinical signs shown in heterozygous positive animals, so the presence of this disorder can only be

detected at birth of an affected foal, unless the mating pair are screened before breeding. Genetic testing of both sire and dam before breeding and ongoing responsible breeding is essential to reduce the likelihood of this disorder. If one of the parents are carrying the gene and the other is free, the foal has a 50% chance of becoming a carrier. If both sire and dam are carrying the mutation, there is a 25% probability that the foal will be affected by the disease, 25% that it will be free and 50% that it will be a carrier [5].

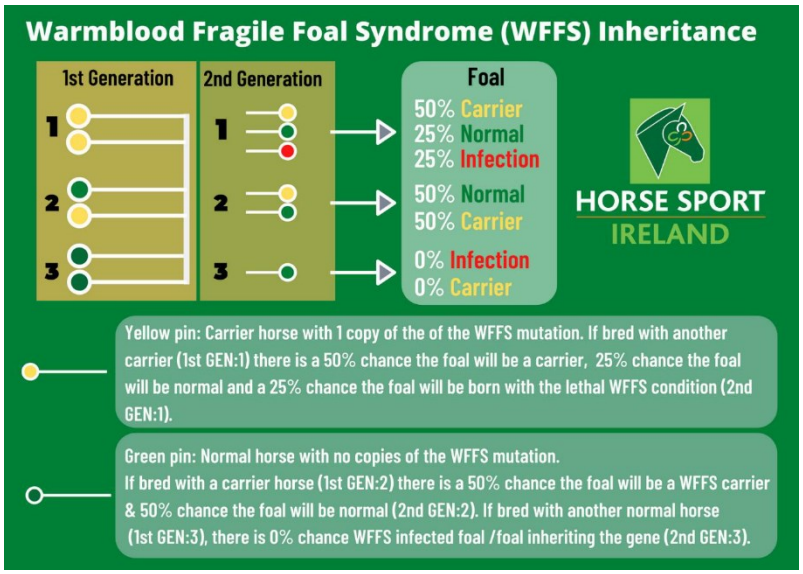


*Figure 1.*

*Inheritance of FFS [5]*

# 2 Objectives

The primary goal of this thesis is to gain a clear and concise understanding of Fragile Foal Syndrome and how it is affecting the equine world today. By delving into the abundance of literature on this specific topic, the goal of this study is to present a comprehensive understanding of FFS itself, and how it has and to this day, continues to, have a practical and economic impact on breeders. The thesis summarises the clinical characteristics associated with FFS by conducting a thorough review into the studies and scientific articles available. With this study, breeders can gain in depth knowledge into FFS, which can highlight the need for genetic screening and in turn, can result in better breeding practice. It has been shown that the use of a qPCR assay to identify the lethal mutation, is a new and faster alternative to any other test available [4]. Again, with the use of genetic screening, this enables breeders to make better-informed breeding decisions. By reducing possible breeding implications, it will also decrease the prevalence of FFS in the long run, avoiding the financial and emotional burden of producing non-viable offspring.

A secondary objective is to investigate the epidemiology of FFS within different horse breeds. As mentioned in the introduction, FFS was once recognised as ‘Warmblood Fragile Foal Syndrome’, however, recent studies have identified the gene mutation in other breeds including Thoroughbreds and Haflingers [6]. This shows that with further exploratory research, more breeds may also be affected. However, this literature review not only analyses the genetic background of FFS but also its corresponding disorders in humans and other species. This is important to highlight as when aiming to shed light on the topic, it can open a window of opportunities for future engagement and collaborations between those involved in human medicine as well as in veterinary medicine. This allows for more knowledge to be shared among the respectable researchers involved, and as a result, create a clear pathway for any future efforts that could lead to possible advancements in genetic comprehension, particularly associated with the PLOD1 gene. The possibility of broader genetic insights can be beneficial for both, the medical world and the breeding world.

In conclusion, this literature review will offer a comprehensive overview of FFS by integrating information from genetic, clinical and breeding viewpoints to guide future equine genetics and breeding management research.

# 3 Literature Review

## 3.1 PLOD1 Gene

The PLOD1 gene, also referred to as procollagen-lysine,2-oxoglutarate 5-dioxygenase 1, is located on chromosome 1p36.2-36.3 [7]. PLOD1 plays a crucial role and so, is essential in the biosynthesis of collagen, more specifically, in the post-translational modification of collagen molecules. Collagen is the most common and widespread protein in the body. There are fibrillar collagens and non-fibrillar collagens. The most typical collagen is type 1 collagen, which can be found abundantly in tendons, bone, skin etc. It is present in both healthy tissue and cancer. PLOD1 is situated on chromosome 1p36, where it produces the lysyl hydroxylase 1 enzyme (LH1) [8]. In humans, LH1's function is to induce hydroxylation of lysine residues in collagen [9]. The function of the hydroxylation of lysine into hydroxylysine is for the mechanical stabilisation and cross-linking of collagen fibres, which are fundamental elements of the extracellular matrix in numerous types of tissues, including the skin and joints [10].

In recent years, mutations of PLOD1 in humans have been linked to the development and increased aggressiveness of several tumours. In a study investigating the role of PLOD1 in bladder cancer, it was shown that the deviated form of PLOD1 was seen in cases with bladder cancer. This study proved a correlation between prognosis and PLOD1 expression. Samples with an elevated expression of PLOD1 were more likely to be associated with a poor prognosis. With the use of 2,2'-dipyridyl in vitro, which is a PLOD1 inhibitor, it suppressed the bladder cancer cell aggressiveness, showing that PLOD1 does play a role in the aetiology of bladder cancer [11]. It has also been confirmed that there was a shorter mean survival time of patients with glioblastomas who have a high number of PLOD1. It was also observed that PLOD1's effects on tumour proliferation thrived in the presence of an environment with low oxygen levels [12]. High PLOD1 expression in pancreatic and gastric cancer has also been shown to affect the outcome and signify poor prognosis for the patient [13–15]. The increase in the number of articles and studies investigating PLOD1's significant effect in the malignancy of tumours represents promising progress and a step in the right

direction for the medical community. It highlights the importance for continued study on this topic, for both, a more in depth understanding and insight of oncology and also, as a result, for the possibility to advance and expand the therapies already in place for cancer. This may lead to the development of potential treatments that target these PLOD1's and inhibit them, potentially resulting in more effective approaches in the future.

## 3.2 Ehlers Danlos Syndrome

Ehlers Danlos Syndrome comprises 13 subtypes of rare inherited disorders. In humans, the kyphoscoliotic EDS (type VI) is caused by a mutation of either the PLOD1 gene or of the FKBP14 gene [16]. More than 20 mutations in the PLOD1 gene have been found to be associated with the kyphoscoliotic form [1]. Clinically, patients are indistinguishable between the 2 forms as they both are phenotypically the same- skin hyperextensibility and bruising, muscle hypotonia, kyphoscoliosis etc. These clinical features are forming due to the dysfunction in the PLOD1 and FKBP14 mechanism of collagen, resulting in, for example, sparse intramuscular connective tissue and distensible joints. FKBP14 can also cause congenital hearing impairment [17]. Currently, there are no treatments for kyphoscoliotic EDS, only symptomatic treatment. However, both PLOD1 and FKBP14 each have distinct molecular profiles, giving a more clear opportunity and understanding of these forms, which could lead to future targeted therapies.

Ehlers Danlos Syndrome has also been discovered in dogs first in 1943, with later findings in cats. Clinical signs primarily affect the skin, with the common clinical signs in humans such as joint laxity, rarely seen in dogs and life threatening systemic signs such as rupturing of vessels, not being reported in dogs or cats [18]. The specific type of Ehlers Danlos Syndrome that affects ruminants is type VIIC, caused by altered ADAMTS2 gene [19]. In dogs, the COL5A1 gene mutation was recognised. This is what is described as 'Classical Ehlers Danlos Syndrome' in humans [20]. We also refer to 'dermatosparaxis' or 'cutaneous asthenia' when describing the skin disorder in animals. In human medicine, the term 'cutaneous asthenia' is not used to describe Ehlers Danlos Syndrome due to it being reserved for

situations where there are defects found in both collagen and elastin, however, in animals, this has not yet been documented [21].

## 3.3 Overview of Equine Genetics

### 3.3.1 Monogenic Disorders

Genetic disorders can be categorised as monogenic or polygenic, depending on the number of genes involved. Mutations in a single gene result in monogenic disorders. These conditions can be classified as autosomal dominant, autosomal recessive or X-linked. It is generally easier to recognise monogenic disorders due to only one gene being altered, and straightforward to avoid passing on the mutated gene to offspring with the use of genetic testing to identify carriers.

Monogenic autosomal dominant means that this pattern only requires one copy of the defective gene to cause the disorder i.e one mutated gene and one normal gene is enough for the horse to be affected with the disorder in question. This results in a 50% chance that offspring will inherit the disorder if one of the parents are affected, and a 100% probability of being affected if one parent is homozygous. Hyperkaliaemic Periodic Paralysis (HYPP) is an example of a monogenic disorder with autosomal dominant inheritance that affects sodium channels, resulting in excess potassium in the blood [22]. It affects mainly Quarter horses and American Paint horses, with sporadic clinical signs such as hypotonia and paralysis. Horses homozygous for HYPP can exhibit symptoms within the first week of life, with more severe symptoms such as respiratory distress or dysphagia. Heterozygous horses tend to show signs after weaning and appear to be affected to a lesser extent. Horses can be screened for HYPP by submitting hair from the tail or mane to a licensed laboratory [23]. Hair samples must include the root for the purpose of genotyping [24].

Monogenic autosomal recessive disorders require two copies of the altered gene, meaning, both parents need to at least be carriers. For example, if both parents are carriers, the offspring have a 25% chance of being affected, 25% that both copies are normal, and a 50% chance that it will be a carrier. So, if the horse only has one mutated gene, and the other is normal, then it will be

labelled as a carrier. Severe Combined Immunodeficiency (SCID) is a common disorder found in Arabian and Arabian crossbred foals, affecting both B and T cells. The absence of these essential lymphocytes results in foals being affected after the period of passive maternal immunity. Once these antibodies from the mother have dropped, foals become extremely susceptible to infections and even with veterinarian care, most die by the age of 5 months [25]. There has been one case of curing SCID with bone marrow and thymic cell transplantation. The experimental transplantation was done using a healthy full sibling, with results stating that when reviewing the foal nearly 1 year post surgery, the foal showed promising results [26]. Genetic testing is essential to prevent the spreading of this disorder to offspring.

Finally, X-linked disorders only affect the X chromosome, so males are more likely to be affected [27]. An example of an X-linked disorder would be Androgen Insensitivity Syndrome, which appears as horses whose genetic sex chromosomes are different to their phenotype [28].

### 3.3.2 Polygenic Disorders

Polygenic disorders are more complex than monogenic disorders, characterised by the involvement of multiple genes contributing to a single phenotype. Polygenic disorders arise from the combined effects of variations in several genes, often impacted by environmental factors, which makes them particularly challenging. For example, polymorphisms in the IHH gene have been connected to osteochondrosis in horses, however, its expression is influenced by additional genetic and environmental variables [29].

The significance of inbreeding and genetic variety in polygenic disorders adds to their complexity. Studies show that there is a connection between genetic predisposition and breeding techniques by demonstrating how high inbreeding coefficients could heighten the risk of specific illnesses such as Club Foot in Arabian horses [30] Breeding plans intended to lower the prevalence of polygenic disorders are further complicated by the considerable variation in the heritability of variables linked to these conditions. For instance, fractures are common injuries for racehorses but the cause of these injuries are not only aggravated by the physical demands of racing, but also linked to genetic factors [31]. This is why it is necessary to

approach polygenic disorders with an open approach, taking into consideration both environmental and genetic factors.

## 3.4 Genetic Testing

### 3.4.1 History

The study of equine genetics has progressed markedly over the last century. It has evolved from basic breeding strategies to revolutionary genomic research. Every day we are learning more, keeping up to date with current trends and also improving wherever we can. There have been outstanding discoveries in recent years with technology that have moulded our interpretation and knowledge of equine breeding.

Since as far back as the domestication of the horse, people were selecting horses based on desirable attributes such as strength, speed and temperament. The domestication of horses led to what would be the start of the ever changing and progressing ways of transport, combat and communication [32]. In war, soldiers needed athletic horses with great stamina, farmers needed heavy, strong built horses to plough the fields- this selective breeding is a result of the diversity of today's horse breeds. However, since domestication, due to the strategic breeding for certain phenotypes, the diversity has reduced. With the introduction of studbooks and widespread selective breeding during the past 200 years, genetic diversity in the modern horse has decreased by 16% [33].

The investigations of Mendelian traits in the horse go as far back as the early 20$^{th}$ century. It started with interpreting the different pigmentations available in horses, with Alfred Sturtevant and Thomas Hunt Morgan, being one of the first people to publish an article on the inheritance of coat colour in harness horses [34]. However, it wasn't until nearly 100 years that the genetic mechanism hypothesised by Sturtevant for the chestnut coat colour was discovered. Other variants influencing pigmentation were also discovered using candidate gene approaches [35]. This approach involves evaluating the relationship between an allele (or set of alleles) of a gene that could be linked to the disease (i.e a candidate gene) and the disease itself. It is imperative that a suitable candidate gene is chosen that could be relevant to the disease in

question, for this approach to work [36]. There are, at present, 58 pigmentation related variations known, 27 of which are found in the KIT gene and contribute to the dominant white phenotype. These discoveries were made possible by the use of readily available SNP array technologies and the high quality of the horse reference genome sequence [35].

Clinical cytogenic research in horses has been underway for more than half a century, with the peak of it being during the 1970-1990s. Researchers discovered, by investigating horse chromosomes, the equine karyotype, which has 64 chromosomes [37]. During this time, they also identified chromosome irregularities that could impact both growth and fertility, which would be important in genetic disorders in horses. Chromosomal abnormalities are among the leading non infectious causes of low fertility, infertility and even birth malformations [38]. Clinical cytogenetics is still being used to this day due to the updated advancement, involving not only the original chromosome analysis by banding techniques, but also incorporating the molecular side of genetics. Techniques such as G-banding, DAPI-banding, with NOR-banding and R-banding being mainly used for research purposes [38]. These techniques evolved from human cytogenetics and have been adopted for equids. G-banding is the most common and works by creating a pattern of alternating light and dark bands, enabling bright field microscopy to identify each chromosomal pair [39]. DAPI-banding (4’6-diamidino-2-phenylindole) is similar to G-banding and is done using fluorescence and can be used in combination with fluorescent in situ hybridisation (FISH), to detect signals from DNA probes whilst at the same time, determining the chromosomal band and probe position [40]. FISH is the most commonly used molecular technique in equine cytogenetics. Clones from the horse genomic bacterial artificial chromosome (BAC) are the most widely utilised probe for FISH. Another probe that can be used for FISH are horse chromosome specific paints made by microdissection or chromosome flow sorting, however, BAC clones were employed in every FISH experiment that validated and improved various horse translocations [38]. HYPP was the first genetic disorder to be identified at molecular level in 1992, discovered by the candidate gene approach [41].

### 3.4.2 The Equine Genome Project

A major breakthrough in the field of equine genetics was celebrated in 2009. The creation and publication of the genome sequence of Twilight, a thoroughbred mare, was made possible by the combined efforts of researchers worldwide [42]. As a result of this transformative moment, equine genomics has advanced to a new level, by shaping horse genome studies and contributing to the current revolution in genomics technologies. This has since aided researchers comprehend the genetic foundation of a wide range of traits and diseases, with this knowledge facilitating multiple equine studies [42]. With the equine genome being available for 15 years now, breeders have been able to use genetic data to make better educated judgements. Genetic testing can aid not only in unveiling potential hereditary problems but also in identifying desirable features, which can contribute to healthier and more successful breeding results. As a result, managing and reducing hereditary illnesses and enhancing certain performance characteristics has become more manageable, for both breeders and vets [43].

### 3.4.3 The Future for Equine Genetics

Precision breeding methods, with the use of genomic selection, are expected to dominate the future of equine breeding. Breeders, through the knowledge of the veterinarian, can more accurately anticipate each horse's genetic potential by examining the full genome. The veterinarian must understand these genetic tests so they can inform their client correctly. With the proper use of this tool, there will be a reduction in the risk of genetic diseases and horses with the preferable qualities can be chosen instead [44].

Gene editing with CRISPR-Cas9 technology, offers unparalleled precision in altering the equine genome. With the use of this technology, scientists can have the ability to modify DNA sequences, for example- correcting genetic abnormalities that are the root of hereditary diseases. CRISPR can alter genes and with doing so, improve desired characteristics. However, there could be questions raised regarding the ethical implications and discrimination of gene editing. There is a possibility for CRISPR to have a huge impact on the equine world if it can ensure that such modifications are safe and beneficial [45].

## 3.5 Pathophysiology of Fragile Foal Syndrome

The symptoms of FFS are due to collagen defects in the horse. Collagen is the predominant protein in the extracellular matrix of connective tissues, with its function being both strength and support. It is synthesised by procollagen, where it then undergoes a series of adjustments. Once it has been modified, it will then be released into the extracellular space, where it is processed additionally into mature collagen fibres, which provide a framework, responsible for supporting tissues and organs, contributing to their current properties and durability [46]. Mutations in the PLOD1 gene are the cause FFS, resulting in a disruption in the formation and role of collagen [1]. As of 2011, thanks to ongoing research, a genetic test was developed for FFS [47] and since 2019, warmblood stallions undergoing HSI studbook inspection must be tested in accordance with Horse Sport Ireland, which also voluntarily provides free testing for sport horse foals [48]. The defective collagen causes a combination of problems such as skin tearing, hyperextensible joints and haemorrhaging, seen in foals from birth. There is no cure to these defects, leading to euthanasia if they haven't died within hours already [1].

### 3.5.1 Hereditary Equine Regional Asthenia

Hereditary equine regional dermal asthenia (HERDA), previously known as 'hyperelastosis cutis', is a skin disorder that primarily affects Quarter horses. It is an autosomal recessive inherited disorder that is triggered by a mutation in the Peptidylprolyl isomerase B (PPIB) gene. PPIB is responsible for producing cyclophilin B, which is involved in collagen folding, with its altered form, causing issues similar to that of FFS and EDS. However, foals at birth rarely show signs, with symptoms tending to start around the age of 1.5-2 years old. Lesions mainly on the dorsum such as sloughing skin, ulcers, haematomas/seromas form around the age that they are broken in, with reports of poor wound healing [49]. An example of this can be seen in Figure 2.

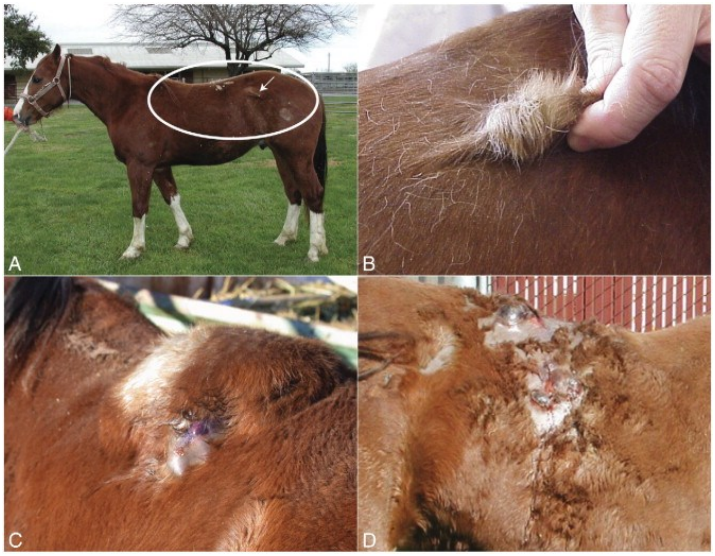


*Figure 2.*

*Clinical Symptoms of HERDA* [49].

However, according to a report published in 2004, a number of horses affected by HERDA were castrated, with their recoveries being without complications [50]. Collagen formation appears to be normal in unaffected parts of the affected horses, deeming these as healthy tissues. Histologically, in affected tissues, the collagen appears to be clustered and disorganised. [49] An article about the effect of solar irradiation on HERDA affected horses was published in 2022, discovered that there is an upregulation of skin collagenase genes such as MMP1, MMP8 and MMP13, in the dorsal skin of horses that have been exposed to the sun. The MMP1 gene was particularly increased with sun exposure for three days. Collagenases are responsible for the breakdown of collagen, resulting in HERDA affected horses with more severe lesions. These findings show that with sun exposure (most commonly on the dorsal skin of the horse), the increase of collagenases can result in the amplification of clinical signs of HERDA affected horses [51].

HERDA, FFS and EDS are all disorders affecting the skin due to a mutation, leading to a disruption in collagen synthesis. Although these three disorders share some similarities, they do not share the same mutated gene, with HERDA being caused by a mutation in the PPIB gene [49], FFS with the PLOD1 gene [1] and the kyphoscoliotic form of EDS forming due to either a mutation in the PLOD1 or FKBP14 gene [16]. Both FFS and HERDA are

affecting horses, with EDS responsible mainly for human disorders (with reports of other animals such as dogs and cats [18]). There is no cure for any of these disorders, so genetic testing has been the golden tool used to identify both carriers and affected individuals. All breeding stallions are required to undergo HERDA testing by The American Quarter Horse Association [52]. In the case of HERDA, most of the horses are euthanised due to not being rideable and not suitable for breeding [53]. Due to being autosomal recessive, the disorder requires both parents at least carry the altered gene for a 25% chance of producing an affected foal. The prevalence of HERDA varies depending on where they are based, with a prevalence of carrier frequency seen at 3.5% in the USA and in France, with a lower figure of 1.6% [54]. HERDA is a relatively rare disorder, with it appearing in China for the first time in 2017 [55].

### 3.5.2 Naked Foal Syndrome

The Akhal Teke horse breed originated in Central Asia and is greatly recognised for its distinctive metallic coat shine and excellent endurance capabilities [56]. The studbook for Akhal Teke's has been closed since 1932. There is a high degree of inbreeding due to the limited gene pool and line breeding practices, which encourages the emergence of recessive genetic diseases [57].

Naked Foal Syndrome (NFS) is a monogenic autosomal recessive trait, caused by the ST14:c.388G.T variant on chromosome 7 and 27 in equines. The variant causes the shortening of more than 80% of the open reading frame of the ST14 gene. It implicates the Akhal Teke breed, with the affected horses having little to no hair, as seen in Figure 3, being exposed to damage without the usual protection of their coat, with all cases so far only surviving from a few weeks up to 3 years old, however it is not known if the premature deaths are a result of a particular pathology. In humans, mutations of the ST14 gene results in ichthyosis. The first recorded case of NFS was in 1938 and since then, the number of cases have progressed. Histopathology of 2 NFS affected horses, 1 carrier and 1 control was done using samples of skin. The hair bulbs of most of the follicles were situated at or just below the sebaceous glands, and both affected horses had significantly shortened

anagen follicles with just fragments of the isthmic part present. The infundibula of the hair follicles were deformed and filled with an excessive amount of infundibular keratin. The sebaceous duct opening was found to be rich in sebum, with the follicular lumen enlarged. If there was any hair present, the shafts were extremely thin and unstructured.

The skin of the affected foals was dry, with the main body of the horse being alopecic, including the mane, tail and eyelashes. In one of the cases, a necropsy was performed which found dysplasia of the tricuspid valve and internal hydrocephalus. The foal was killed at 21 days old due to an idiopathic leg fracture occurring.

So far, the ST14 gene mutation has only been found in Akhal Teke horses, with only one article published, to date, on this specific mutation [58].

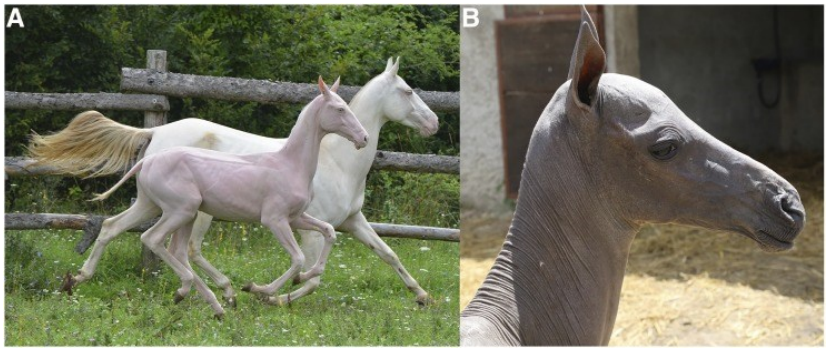


*Figure 3.*

*Foal with NFS* [58]

## 3.6 Fragile Foal Syndrome

### 3.6.1 History of Fragile Foal Syndrome

Warmbloods are groups of breeds, most commonly developing when breeding Thoroughbreds and Arabians with draft horses. With the broad history of warmbloods, this makes identifying the original founder of FFS more complex. Conspiracies implicating both Arabian and Thoroughbred horses have been circulating, one being, that a thoroughbred known as Dark Ronald, could be the source. Dark Ronald was an English thoroughbred stallion, an ancestor of many German warmbloods, who lived from 1905, until 1928 where he was transported to a veterinary hospital due to colic and died. His body was preserved using formalin for the heart and skeleton and

dehydrating the skin and to this date, remains in the Museum of Domesticated Animals of the Central Natural Science Collections of Martin-Luther-University Halle-Wittenberg. With these preserved remains, it was possible to isolate the DNA of the skin to determine the position of the PLOD1 gene. By the use of this method, it was shown that Dark Ronald, was not the cause of the FFS disorder [59].

Another hypothesis is that, Bairactar Or. Ar, a famous Arabian stallion who was imported in 1817 from the Middle East to a stud farm in Weil, Germany, is the source of FFS due to many of his progeny having symptoms that correlate with FFS. Foals were born alive and had skin lesions found on the legs and back. They also could be found with flexed forelimbs [60]. To this day, many of his descendants are not only in pure Arabian bloodlines, but also present in warmblood lines. He produced elusive progeny such as Amurath Sahib, a well known Arabian stallion used in Poland. There are no remains of Amurath Sahib, however, the skeleton of Bairactar Or. Ar is located in the Stud Museum Offenhausen in Germany. DNA was extracted from his tooth and analysed but did not find a mutation of the PLOD1 gene, with the reference allele, the G-allele, which is the reference allele, being at the c.2032 position [6].

To this day, the founder of the missense mutation of the PLOD1 gene that causes FFS is still unknown. In a study done in 2020, they detected the most recent common ancestor of a select number of tested genetic carriers in Europe. 81 genetic carriers of FFS had their pedigrees analysed and compared, resulting in this common stallion, who was born in 1861. This common ancestor was of a traditional sire line F/W in the Hanoverian. It is thought that the variant can be traced even further back to a thoroughbred, due to the 38.4% of thoroughbred found in the Hanoverian population. However, this has not been proven yet and remains a theory, rather than fact [61]. Due to the extensive number of breeds involved across the world, and other species such as humans, dogs, cattle being affected with similar symptoms [16, 18], the mutation's origin is much more complex. Assuming the mutation is linked to one line could be looking at it in the wrong light. With the co-operation of researchers, vets and breeders all around the world,

the pedigree of other influential stallions can be traced back and analysed, which allows for a broader pedigree database.

### 3.6.2 Clinical Signs and Symptoms

Only horses with two copies of the mutated gene are prone to clinical signs. In order for this to occur, both parents must be carriers, resulting in a 25% probability that the foal will be homozygous, showing clinical signs, 25% that it will be negative and so, unaffected and 50% chance that it will be a heterozygous carrier. The tragic effects that occur in an affected foal, ultimately end up in euthanasia within a few hours.

There have only been 19 cases reported of FFS to date [62]. Cases have reported foals with a range of symptoms such as having hyperextensible and hypermobile joints as well as fragile and easily tearing skin. Joints have also been found to be contracted, resulting in the inability to extend. Some cases have found hemarthrosis of the joints, particularly of the shoulder and elbow [62]. These deformities lead to problems both in-utero and post foaling. Abnormal limb positions in-utero result in dystocias and an increased risk of joint injuries and when born, the hyperextensible or excessive flexing of the joints can affect their movement, leading to further musculoskeletal problems. Both the joints and skin being affected often results in a poor prognosis for the affected foals, leading to death, most commonly via euthanasia [4, 60]. Similar clinical signs were first reported in 1892 in humans, with it later being referred to as Ehler Danlos Syndrome [1]. With homozygous foals being born at a mature age, it can be assumed that if any homozygous foals are terminated during pregnancy, it is most likely closer to term than in the embryonic stages. A 24 year long study was conducted in France on equine abortion, where it found that in foetal abortions, 50% are infectious related, 25% of non-infectious origin and the last 25%, with no diagnosis. FFS is not included in routine diagnostics for abortion, so, a number of cases could be a result of FFS, but have gone unnoticed. This could explain the discrepancy in the prevalence of the disease and the number of cases reported. In the 24 year long study, 52 cases of abortion were analysed and 14 warmblood pregnancies were homozygous positive. Homozygous foals were born from month 9 to month 11, born alive but non-viable or born

dead. Among the 14 foals with FFS, five of them required assistance at birth, such as a caesarean section and foetotomy. The dystocias that occurred were mainly due to flexed forelimbs, with one foal having incorrect positioning of the head. Apart from the common findings in FFS, one of the foals was found with a prolapse of the small intestines due to incomplete closure of the abdominal wall. Another foal was found with several perforating lesions of the aorta and vena cava. It also had appeared with a haemorrhage on the left side of the thorax, intramuscularly. Spinal scoliosis was also a finding in one of the 14 foals. Five of the foals were sent for necropsy, with two foals having further haemorrhaging intracranially. None of the mares with homozygous foals had been tested for FFS before breeding, however, the stallions bred to these mares were heterozygous for FFS [63].

The severe fragility of the skin is one of the main characteristics of FFS. Foals affected by FFS often have thin, easily damaged skin, which result in open wounds (see Figure 4 below). These cutaneous wounds are then exposed to the environment, which can cause impaired wound healing and further infection [62, 64]. However, the foals tend to die within hours of life. The skin lesions are not only superficial but can also extend to deeper layers, resulting in hematomas [64]. These defects in the skin layers are due to collagen dysplasia, damaging the skin's integrity [62].

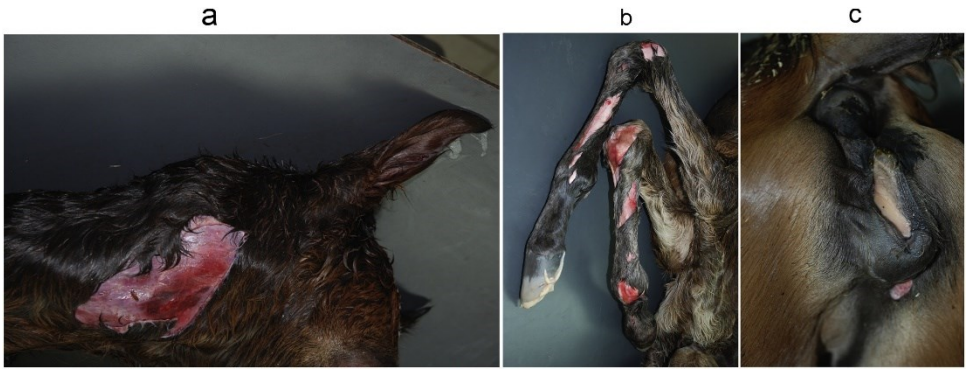


*Figure 4.*

*Skin defects found on the head, limbs and vulva in foal with FFS* [60].

### 3.6.3 Prevalence

The prevalence of fragile foal syndrome is notably higher in warmbloods than in other types of horses. Originally, the disorder was known as Warmblood Fragile Foal Syndrome but since emerging cases of other breeds

involved, has been more commonly known as Fragile Foal Syndrome. In a study done in 2020, with both, the Hanoverian and the Danish Warmblood having a carrier prevalence of 17%. A number of American warmbloods were also tested, with a carrier frequency of 14%. An unusual finding was that two Haflingers that were tested were found with a single copy of the mutated allele. This could be due to the fact to their origin being Europe and their studbook not being closed until 1946. This would allow time for influence by other breeds, such as European warmbloods or Thoroughbreds, who would be more likely to carry the missense mutation [6]. Many studies have been conducted on the prevalence of FFS in Europe, especially in Germany and Sweden, which comes to no surprise as the majority of warmbloods originated from Germany. It was reported that 7.4% of Swedish Warmbloods are estimated to be carriers of the mutation of the PLOD1 gene [64]. Given that homozygous individuals usually have severe clinical indications or are not viable, this raises concerns about the possibility of producing diseased foals.

The first recorded case of fragile foal syndrome in a homozygous thoroughbred foal was discovered in 2022. The mare presented to the clinic one month prior to the delivery of the homozygous foal. The mare presented with “premature mammary development, a premature rise in serum progesterone” and upon an ultrasonographic exam, an abnormal finding on the neck of the foetus was found, which later was discovered to be a hematoma. The mare was admitted to the hospital where she was anaesthetised and foaled on day 309 of gestation with controlled vaginal delivery. The live foal displayed clinical signs such as haematomas on the neck as well as fragile skin. Not only were the skin and joints affected, but the bones too. A spinal scoliosis was discovered, as well as deviations of the maxillary and frontal bones. Due to these devastating clinical symptoms, the foal was euthanised shortly after birth. The case was confirmed by both whole genome sequencing and commercially available genotyping. DNA was taken from the gluteal muscle and assessed using spectrophotometry. This DNA was tested for both HERDA and FFS using the commercial assays. A post mortem was performed with samples of the skin and tissue taken [62]. The prevalence of the Thoroughbred was reported to be 2.37% [6]. This

figure, although not as high as in warmbloods, still justifies genetic screening being done before breeding.

### 3.6.4 Diagnostics

The foundation of diagnosing FFS lies in genetic testing by detecting the presence of the PLOD1 gene mutation. To identify the precise single nucleotide polymorphism (SNP) that causes FFS, a quantitative PCR assay can be used. The University of College, Dublin, used the qPCR method as opposed to the more costly two step PCR and Sanger sequencing that are being used. Samples are taken from the hair of the horse, making sure to include the root. The DNA was extracted by using the Qiagen DNA blood and tissue kit. Ten hair strands per extraction to 1cm of length are used and put into a 1.5ml microcentrifuge tube that was free of RNase and DNase. Next, 300 microlitres of ATL buffer, 20 microlitres of proteinase K and 20 microlitres of a 1 M DTT solution were added, followed by vortexing the sample while incubated at 56 degrees Celsius until the sample is fully lysed and can no longer be seen anymore [4]. ATL is a buffer for lysis, containing both sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic acid (EDTA) and responsible for purification of nucleic acids. The proteinase K is an enzyme whose function is to breakdown cellular proteins [65]. The sample is then vortexed for a further 15 seconds to guarantee total homogenisation. 300 microlitres of AL buffer is then added before undergoing another vortex. 300 microlitres of pure ethanol is added and vortexed. The sample is then centrifuged with additions of more buffers, ensuring after each centrifugation, that the flow through is removed.

To amplify the area of equine PLOD1 containing the desired SNP, two primers were created, ensuring that they would not amplify human PLOD1 to prevent any contamination issues. Additionally, two allele specific probes were developed to bind specifically to the SNP's location. The wild type sequence was matched by one of the probes, while the mutated variant was identified by the other. Each probe contained a distinct fluorescent dye (FAM-mutant and VIC-wild type) and a quencher (TAMRA) attached. The idea of the assay is that if the wild type sequence is present during the PCR cycle, the subsequent wild type probe will bind to it. During amplification, a

signal will be produced when the DNA polymerase interacts with the probe, which will in turn, knock off the fluorescent dye and release it from the quencher and vice versa with the mutant specific probe. This assay can accurately determine if a sample is either homozygous wild type or heterozygous. This newly developed PCR based assay has been validated by the one company in Ireland who performs the genetic test for FFS, labelling it as the 'gold standard'. This new assay is more cost efficient and rapid than any other test on the market [4], possibly making it more desirable for breeders to use in the future.

Sanger sequencing, also known as the chain termination method, was first developed in 197 and since then, has played a crucial role in molecular biology. Firstly, the PCR is used to amplify the DNA, followed by a sequencing reaction with the addition of fluorescently labelled dideoxynucleotides (ddNTPs). These ddNTPs end DNA strand elongation at particular bases, allowing fragments of different lengths to be generated. These fragments undergo capillary electrophoresis, which is responsible for separating them based on their size, allowing the sequence to be read using the fluorescent signals. Sanger sequencing is reliable and highly accurate, however, it is expensive and not suitable for larger-scale undertakings, like the sequencing of a complete genome [66]. Genetic research has been transformed by next generation sequencing (NGS). Multiple genes may be sequenced simultaneously, enabling researchers to a more thorough understanding of genetic changes across populations. It is particularly beneficial for finding unusual variants and comprehending the genetic makeup of complicated features [67, 68].

### 3.6.5 Prevention and Management

Unfortunately, there are no treatment options for homozygous foals, so the only way to manage the disease is to prevent it from occurring. This can only be done by screening both mare and stallion before breeding to ensure that both of them are not carriers. Due to the recessive nature of the disorder, the foal requires two copies of the altered allele, which means that both parents need to be heterozygous for the disorder, for a 25% chance that they will produce a homozygous foal. If, for example, a mare is a carrier for the

PLOD1 mutation, and the stallion is negative, breeding can occur without risk of producing a foal with the disease, however, there will be a 50% chance that it will be a carrier. Ideally, it is desirable to produce progeny that are negative to FFS to help eliminate the disorder completely however, this is not always an option if producing top quality foals.

It has been shown that the public's perspectives on genetic testing are influenced by their understanding of genetics [69]. It is important for vets to have a clear insight on genetics to be able to explain and be clear and concise to breeders in order for them to make knowledgeable decisions. The author considers that a possible problem that may occur is the apprehensiveness of breeders to test their horses. This is likely in fear of finding out they are carriers of mutated genes. Many breeders could be concerned that this potential information would have a negative impact on their horses' market value and possibly limit their breeding opportunities, thus, resulting in economic losses. This could put pressure on breeders to modify their breeding operations and spend more money on genetic screening of other horses, especially if one of their horses is found to be a carrier. If one of their horses is tested and found to be a carrier, the breeder will need to invest more money into assuring that the horse bred with their horse is not a carrier to eliminate any chance of producing a homozygous foal. A horse known to be a carrier of an altered gene could also be more difficult to sell due to the stigma around carrying genetic abnormalities.

Furthermore, breeders may be hesitant to pursue testing due to concerns about negative publicity surrounding hereditary illnesses. They can be concerned that disclosing their carrier status will cause negative publicity, less interest in their horses, or even harm their reputation in the equine world. The advantages of preventative genetic testing should be communicated to breeders in a more proactive way, so as to highlight that the benefits outweigh any potential negative outcomes.

# 4 Materials and Methods

## 4.1 Search Strategies

An extensive examination of the available literature was completed in order to compile relevant articles regarding fragile foal syndrome. Databases such as Google Scholar and PubMed were used to gather the relevant information, mainly from articles published between 2000 and 2024. Keywords such as "Fragile Foal Syndrome", "Ehler Danlos Syndrome" and "equine genetic disorders" were used.

## 4.2 Screening and Selection of Research

The initial search for scientific papers relating to this thesis produced more than 90 relevant results. Subsequent reviews of these papers led to the inclusion of 69 papers for the final analysis. Data covering topics such as clinical presentations and diagnostics were extracted from these papers and analysed before combining the applicable information into this thesis.

## 4.3 Limitations of Research

Only English language articles were included in the review and a small number of potentially relevant articles were unavailable due to subscription restrictions. One significant limitation of the study is the sample size, with only 19 cases of Fragile Foal Syndrome to date being recorded. This small sample size limits the data that can be drawn from the research. A greater sample size would improve the value of the research.

## 4.4 Interview with a molecular geneticist

On the 23rd February 2024, I attended a meeting with Dr. Tosso Leeb, of the University of Bern, Institute of Bern, to discuss autosomal recessive disorders in equines. He offered insight into his recent paper on Naked Foal Syndrome in Akhal Teke horses. It was beneficial for the purpose of this review, to draw comparisons between Naked Foal Syndrome and Fragile Foal Syndrome, both autosomal recessive disorders of the skin. He outlined the practical approach to diagnosing such disorders.

# 5 Results

This literature review provides insights into Fragile Foal Syndrome and how it effects the breeding world of horses.

## 5.1 Genetic background of FFS

A mutation in the PLOD1 gene, whose role is primarily collagen synthesis, is responsible for FFS. This variant results in the disruption of collagen cross linking, causing structural instability in connective tissues. Due to the nature of the disease, it requires two copies of the mutation, one from each parent, in order for the offspring to be affected. Despite originally being identified as Warmblood Fragile Foal Syndrome, it is not exclusive to warmbloods as it has recently been discovered in other breeds such as Thoroughbreds and Haflingers, so is now more commonly referred to as Fragile Foal Syndrome.

## 5.2 Clinical Symptoms

FFS affected foals possess severe abnormalities such as hyperextensible and frail, easily torn skin and laxity of joints, which frequently results in wounds, joint dislocations and mobility difficulties. Due to the severity of these malformations, foals die within hours or are euthanised to prevent further suffering. These clinical signs can be seen at birth, with anomalies such as scoliosis, haematomas and joint contractures causing dystocias at delivery.

## 5.3 Genetic Carriers

Warmblood horses are the most common carriers of FFS, with some studies highlighting a carrier rate of 17% in Danish warmbloods and Hanoverians. Thoroughbreds have a lower genetic prevalence of 2.37%, however this is still concerning and emphasis the requirement of genetic testing in these breeds also. Haflingers are known to carry the genetic mutation also, but it is much less common in these horses and its presence is presumed to be due to breed crossings with warmblood lines in the past.

## 5.4 Genetic Testing

This thesis also highlighted a number of diagnostic methods for FFS. The implementation of a qPCR assay to detect the SNP in the PLOD1 gene has drastically improved the diagnosis of FFS due to it being a cost-effective, fast and reliable means of identifying carriers. Compared to some traditional methods, such as Sanger sequencing, this diagnostic method offers early diagnosis and subsequently, the prevention of possible future cases, allowing keepers to make more informed breeding decisions.

## 5.5 Economic and Ethical Impacts

There is a profound economic impact for a breeder when a foal is born with FFS. As well as the emotional costs to the breeder, the euthanasia of non-viable foals, as well as veterinary care and medications for the mare can result in significant financial losses. This further underscored the necessity of genetic screening. While breeding two carrier animals has the potential to produce offspring that are capable of becoming high-performance horses, it also increases the risk of producing foals affected by FFS, thus raising ethical questions that need to be addressed further.

## 5.6 FFS Compared With EDS

An analysis of the commonalities and the differences between FFS in horses and EDS in humans or animals serves to create an understanding of the impact of the mutation across different species. Humans tend to experience milder symptoms compared to horses, who are often euthanised due to the severity of the defective collagen synthesis, even though both disorders are caused by mutations in the PLOD1 gene.

## 5.7 FFS Compared With HERDA

This thesis also investigated the similarities between FFS and HERDA, which is a disorder of the collagen in Quarter Horses. Unlike FFS, HERDA comprises of a different genetic origin, PPIB mutation and horses affected by it display a later onset of clinical signs. This resulted in a deeper

understanding of how crucial it is to implement genetic testing that is specific for each disorder, based on the breed of the horse.

# 6 Discussion

The findings of this thesis highlights the significant effects that Fragile Foal Syndrome (FFS) has on equine welfare, breeding practices and genetics. The cause of this disorder is due to a mutation in the PLOD1 gene, impairing collagen synthesis, which results in severe connective tissue issues. Other connective tissue disorders that mirror similar pathophysiological characteristics to FFS include Hereditary Equine Regional Dermal Asthenia (HERDA), mostly found in Quarter horses, and Ehlers Danlos Syndrome (EDS) in humans. In order to ensure precise diagnosis and prevention, it is imperative to undergo disorder-specific genetic testing. The horses affected by FFS require euthanasia shortly after birth, if not stillborn, which furthermore highlights the need for proactive measures within the equine industry.

The prevalence of FFS among warmblood breeds in particular, ranges from 14-17%, with the mutated gene recently being found in other breeds such as Haflingers and Thoroughbreds. This underscores an urgent need for breeding programs to incorporate genetic testing procedures to avoid the breeding of two carriers. The thesis demonstrates the efficacy of the available diagnostic techniques, such as the qPCR assay, which is a both rapid and affordable method to detect the PLOD1 mutation. However, the stigma attached to horses carrying the mutation and breeders' concerns about possible financial consequences continue to hinder the use of genetic screening.

The management of FFS is further complicated by its ethical and economical aspects. The emotional toll on breeders is devastating, in addition to financial losses from veterinary expenses and euthanasia. This leads to questions emerging regarding the ethical concerns of producing non-viable foals in pursuit of high-performance offspring. In order to address such issues, it is important to encourage responsible breeding practices and aid in educating breeders about important disorders and how they arise.

# 7 Conclusion

Fragile Foal Syndrome (FFS) presents challenges to not only the veterinary medicine world, but also to the genetic and equine industries. This thesis not only emphasises the importance of genetic screening being used to prevent disorders such as FFS, but also the need to further investigate into the genetic underpinnings behind FFS and its epidemiological trends across various breeds. With the use of genetic screening, breeders are provided with the opportunity to mitigate the risks of producing affected foals while still maintaining genetic diversity. Efforts towards raising breeder awareness and resolving economic and ethical concerns surrounding genetic testing should be made. With collaboration between veterinarians and geneticists to advance the diagnostic techniques, the equine industry can take the necessary steps to reduce the prevalence of FFS and improve overall welfare of horses. With similarities to EDS, human medical specialists can also co-operate with veterinarians to better understand and shed light on connective tissue disorders and as a result, improve diagnostic research and therapeutic development, for both human medicine and veterinary medicine.

In summary, FFS has recently become more of a challenge to veterinarians, equine geneticists and the equine breeding world. However, it can also be an opportunity to further advance diagnostic testing and to protect and prioritise the welfare of future progenies by adopting informed breeding practices. Focusing on educating breeders on the impact of genetic disorders occurring due to lack of screening, the stigma around genetic testing can be eliminated and thus, the prevalence and spread of FFS can be reduced significantly.

# 8 Summary

Fragile Foal Syndrome (FFS) is a lethal autosomal recessive genetic disorder, initially found in warmblood breeds, but since found also in other breeds such as Haflingers and Thoroughbreds, raising concerns about its increasing prevalence. This fatal disorder is caused by a mutation in the PLOD1 gene, resulting in the disruption of collagen synthesis. Affected foals show severe signs such as hyperextensible skin and hypermobile joints, resulting in euthanasia shortly after birth. This thesis provides a thorough examination of FFS, including its genetic foundations, clinical presentations and implications for equine breeding and welfare.

This study highlights the genetic underpinnings of FFS, whilst also comparing it to similar disorders such as HERDA and EDS, which also affect collagen structure, resulting in connective tissue fragility. These disorders differ in their genetic origins, which highlights the importance and need for genetic testing to interpret and detect specific disorders, resulting in accurate diagnosis and further appropriate measures. The development of qPCR to detect FFS carriers is an advancement however, breeders remain unsure of genetic testing due to the negative stigma surrounding it in the equine industry. This thesis emphasises the importance of genetic screening to avoid producing positive progeny, which would affect breeders both financially, and emotionally.

In conclusion, this study promotes increased awareness and knowledge of FFS, and its genetic effects, the introduction of advanced diagnostic techniques and the importance of incorporating genetic screening into routing breeding practices. With informed teaching to breeders and encouraging the use of genetic testing, the equine industry can reduce the incidence of FFS and improve the welfare of horses. This will also allow more opportunities for future developments in genetic research. It is not just one party that can reduce the prevalence of this disorders and many others, but cooperation amongst various groups such as veterinarians, geneticists, human doctors and breeders. With interdisciplinary efforts from multiple parties, there are endless opportunities and possibilities to delve deeper into the understanding and thus, prevention of many disorders, affecting both humans and animals.

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# Thesis progress report for veterinary students

Name of student: Savannah-Rose McAuley

Neptun code of the student: Z0WP0G

Name and title of the supervisor: Dr. Andras Gaspardy, Dr. Mihaly Kocs

Department: Animal Breeding and Genetics

Thesis title: Fragile Foal Syndrome: Understanding Its Genetic Basis and Implications for Equine Breeding Practices

**Consultation – 1st semester**

| | Timing | | | Topic / Remarks of the supervisor | Signature of the supervisor |
|---|---|---|---|---|---|
| | year | month | day | | |
| 1. | 2023 | 05 | 13 | Meeting with Dr. Kocs at his Akhal Teke stud farm | Dr. Gáspárdy Dénes |
| 2. | 2023 | 06 | 06 | Signing thesis application form | Dr. Gáspárdy Dénes |
| 3. | 2023 | 11 | 08 | Meeting- Given contacts to email for further information on the Akhal Teke breed | Dr. Gáspárdy Dénes |
| 4. | 2023 | 12 | 02 | Field trip | Dr. Gáspárdy Dénes |
| 5. | 2023 | 12 | 16 | Field trip | Dr. Gáspárdy Dénes |

**Grade achieved at the end of the first semester: 5 (excellent)**

**Consultation – 2nd semester**

| | Timing | | | Topic / Remarks of the supervisor | Signature of the supervisor |
|---|---|---|---|---|---|
| | year | month | day | | |
| 1. | 2024 | 03 | 07 | Meeting with both professors | Dr. Gáspárdy Dénes |

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| | | | | | |
|---|---|---|---|---|---|
| 2. | 2024 | 09 | 3 | Discussing the progress of the thesis | Dr. Farpándy Dalma |
| 3. | 2024 | 10 | 16 | Writing the thesis; beautiful, independent literature collection | Dr. Farpándy Dalma |
| 4. | 2024 | 11 | 15 | Discussing the progress of the thesis | Dr. Farpándy Dalma |
| 5. | 2024 | 11 | 20 | Discussing the final version of thesis | Dr. Farpándy Dalma |

**Grade achieved at the end of the second semester: 5 (excellent)**

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

Dr. Farpándy Dalma
……
signature of the supervisor

Signature of the student: SRUCA……

Signature of the secretary of the department: ……..……

Date of handing the thesis in…… ……..…...

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