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Characterization of abortion, stillbirth and non-viable foals homozygous for the *Warmblood Fragile Foal Syndrome*

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ABSTRACT

Warmblood fragile foal syndrome (WFFS) is a monogenetic defect with autosomal recessive inheritance. The WFFS homozygosity is non-compatible with extra-uterine life. Although as many as 15% of Warmblood horses are WFFS carriers, there has been little veterinary focus on this condition. The aim of this study was to determine outcomes and symptoms of clinical signs and pathological abnormalities during pregnancies when there were WFFS homozygous foetuses. Diagnostic material of 15 abortion or stillbirth cases with suspected diagnosis of WFFS was available for this study. Additionally, there were examinations in 37 cases where there were no indications of WFFS when submitted for routine diagnostic procedures. Foals in all cases were genotyped and external morphological defects were recorded. Amongst the 15 cases in which WFSS was suspected, there were 14 homozygous foetuses with the WFFS allele (WFFS/WFFS). Three heterozygous WFFS foetuses (N/WFFS) were detected in the cases submitted for routine diagnostic procedures. Of the 14 WFFS homozygous foetuses, 11 of mares had a gestation length of at least 320 days. Nine foals were born alive but died within a short time. Skin defects were obvious in 12 WFFS homozygous foals, and there was abnormal flexibility in the digital joints, flexed forelegs and incomplete closure of the abdominal wall in five, four, and one of the foals, respectively. In conclusion, the predominant manifestation of WFFS are death during the latter stages of gestation or live births with foals being non-viable. Losses in Warmblood horse breeding caused by WFFS are greater than previously assumed.

1. Introduction

The Warmblood fragile foal syndrome (WFFS) is a monogenetic defect in horses with autosomal recessive inheritance. The WFFS is characterised by a point mutation of the gene coding for the procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (*PLOD1*). In humans, mutations affecting this gene are one cause of Ehlers Danlos-syndrome, characterized by hyperelasticity of the skin and joints, vascular lesions and cardiac and ocular defects (Burrows, 1999).

A gene test for the WFFS c.2032 G > A mutation of the *PLOD1* gene in horses is available (Winand, 2011) and due to fears of an Ehlers-Danlos-like genetic defect, sport horses throughout the world have been tested for WFFS. In Germany, the country with the

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largest sport horse population in Europe, between 10% and 15% of Warmblood horses are heterozygous and asymptomatic WFFS carriers (Müller-Herbst, unpublished observations). A similar percentage of WFFS heterozygous horses can be assumed for Warmbloods worldwide and has recently been described for Warmblood sport horses in Brazil (Moraes Dias et al., 2019). To the best of our knowledge, no published information on the WFFS allele in other horse populations than the Warmblood exists. Because no single live WFFS homozygous horse older than a few days has been detected with routine screening (Müller-Herbst, unpublished observations), one can conclude that the WFFS homozygous genotype is incompatible with extra-uterine foal viability. Only a single veterinary case report on a WFFS homozygous foal born has been published (Monthoux et al., 2015). Clinical findings in this one foal were characterised by dermal lesions and incomplete closure of the abdominal wall. The foal, therefore, was euthanized immediately after birth. Histological analysis revealed an abnormally thin dermis with a reduction and insufficient interlinking of collagen fibres (Monthoux et al., 2015).

Matings of WFFS carrier mares with carrier stallions will result in 50% of the offspring being heterozygous carriers, 25% negative and 25% homozygous for the WFFS mutation. With 10% of Warmblood horses being WFFS carriers and the assumption of random matings with regard to WFFS, in Germany, the expectation was that there were 62 homozygous foals produced in 2019, based on 31,000 Warmblood mares bred in 2018 and an end of season pregnancy rate of 80% (German Equestrian Federation, 2019). Similar numbers can be expected for other Warmblood populations worldwide. It is thus striking that there is only one publication of a WFFS case (Monthoux et al., 2015). One might assume that foals born with similar clinical findings as to what occurs with WFFS would often be submitted for at least visual veterinary examination and necropsy. This, however, is apparently not the case, therefore, WFFS homozygous offspring have not been evaluated by veterinarians.

Pregnancy losses due to genetic defects may occur at any stage of gestation. Because mature but non-viable WFFS homozygous foals can be born (Monthoux et al., 2015), it is assumed that such pregnancies are not primarily terminated in the embryonic stage but are lost at later stages of gestation. In 50% of foetal abortion cases submitted for laboratory analysis, infectious causes are detected while non-infectious causes make up 25% of the cases and there is no aetiological diagnosis in another 25% (Laugier et al., 2011). With WFFS-testing only performed on a larger scale in adult horses since 2018 and so far not included when conducting routine diagnostic procedures for pregnancy losses, WFFS-related abortions are likely to have gone unnoticed. The present study was based on the hypothesis that there is death of WFFS-homozygous horse foetuses during the latter stages of pregnancy. By assessing only for non-viable foals with the described WFFS-lesions (Monthoux et al., 2015) there may be underestimation of the importance of WFFS-related losses in horse reproduction.

2. Materials and methods

2.1. Animals

A total of 52 cases of mare foetal abortion, stillbirth and birth of non-viable foals were included in the study. Cases were either submitted for diagnostic procedures to the Division of Veterinary Obstetrics at Vetmeduni Vienna, Austria ($n = 3$), the Department of Pathology at the University of Veterinary Medicine, Hannover, Germany ($n = 34$) or were evaluated in cooperation with practicing veterinarians in Germany ($n = 15$). Practicing veterinarians had been contacted by the authors of the present manuscript via veterinary journals and social media to provide cases with a suspected diagnosis of WFFS and/or originating from matings to known WFFS-carrier stallions ($n = 15$). For comparison, foetal abortion cases of mares submitted for routine diagnostic procedures to the Department of Pathology at the University of Veterinary Medicine, Hannover, Germany were included ($n = 37$). The month of gestation when stillbirth or abortion occurred was determined and there were comparisons when there were foals with WFFS/WFFS and all other pregnancies using the Mann Whitney test with the SPSS-IBM statistics programme.

Of the overall 52 cases, 36 were Warmblood pregnancies, 13 were of other breeds and in three cases the breed remained unknown (Table 1). Infectious causes of abortion (Equine Herpesvirus 1 and 4 [EHV], Equine Arteritis Virus [EAV], potentially pathogenic bacteria) were investigated in 24 cases using routine diagnostic procedures.

2.2. Diagnostic procedures

All foetuses were genotyped for the WFFS mutation in the *PLOD1* gene. Foetal material (blood, hair or tissue [ear, tongue, liver or lung]) was submitted to Laboklin after collection. The DNA was isolated from foetal material either by using the MagnaPure 96 kit (Hoffmann-La Roche; Basel, Switzerland) or the QIAamp® DNA Micro Kit (Qiagen, Hilden, Germany), following the manufacturer´s instructions. For the determination of the genotype (N/N, N/WFFS, WFFS/WFFS) of the respective c.2032 G > A mutation in the *PLOD1* gene (Winand, 2011), a TaqMan SNP Assay (Applied Biosystems, Waltham, MA, USA) was performed using the TaqMan SNP Genotyping Assays User Guide, utilising FastStart Essential DNA Probes Master and LightCycler 480 II (Hoffmann-La Roche). Primers and specific probes for the detection of the variants c.2032 G (wildtype allele, N) or C.2032A (WFFS allele, WFFS) were designed according to instructions from the test developers and patent holders. Laboklin is licensee of the respective patent.

2.3. Clinical appearance and pathology

For the 14 cases for which the genotype WFFS/WFFS, morphological descriptions and photographs were provided by the veterinarians that consulted with the horse owners. Of these cases, five were submitted to necropsy (Foals 1, 6, 12, 13, 14, Table 1) and a skin sample was provided for patho-histology in one case (Foal 3, Table 1). Tissue samples for histological examination were fixed

Table 1
History and diagnostic findings in foals (n = 52) submitted to necropsy because of abortion, stillbirth or neonatal death.

No	Breed	WFFS genotype	Stage of pregnancy (crown-rump-length, weight)	Foal death	Skin lesions	Further lesions and findings	Microbiology
A. Foals with a suspected diagnosis of WFFS							
1	Warmblood	WFFS/WFFS	term (110 cm, 48 kg)	born alive	Multiple skin defects on limbs, neck and vulva,	Intraspinal and intracranial haemorrhage (8 cm in diameter), pulmonary dystelectasis	EAV neg. EHV1/4 neg.
2	Warmblood	WFFS/WFFS	5 weeks preterm	born alive	Skin defects on one front leg	Abnormal flexibility of digital joints, open abdominal wall. Ten days before foaling udder development and placental separation	
3	Warmblood	WFFS/WFFS	6 weeks preterm		No obvious external lesions but skin "atypically thin".	Premature udder development and loss of milk	
4	Warmblood	WFFS/WFFS	3 weeks preterm		Multiple skin defects	Stiff carpal joints, abnormally flexible digital joints, deformed spinal cord, "googly eyes"	
5	Warmblood	WFFS/WFFS	term (d 335) "big foal"	died 2 min after birth	Two skin defects ventral at abdomen, one 25-30 cm skin lesion on neck	Dystocia (dorsoventral position, deviated head)	
6	Warmblood	WFFS/WFFS	term (117 cm, 38 kg)	born dead	Skin defects at obstetrical manipulation, fragile, thin skin, only loosely attached to subcutis,	Dystocia (malpresentation) Pulmonary atelectasis	EAV neg. EHV1/4 neg.
7	Warmblood	WFFS/WFFS	day 320		No obvious external defects		
8	Warmblood	WFFS/WFFS	day 336	alive before fetotomy	Multiple skin defects approx. 5 cm in diameter medial on right front leg.	Dystocia (deviated head, partial fetotomy)	
9	Warmblood	WFFS/WFFS	2 weeks preterm	born alive, euthanised	Multiple skin defects		
10	Warmblood	WFFS/WFFS	8 weeks preterm		Skin defects on bone protuberances		
11	Warmblood	WFFS/WFFS	term	born alive, euthanized	Distal half of tail without hair	Stiff carpal joints, no fetlock bone palpable in hindlegs, "hoof rotatable in skin", Dystocia, neonatal reflexes normal, no attempt to stand	
12	Warmblood	WFFS/WFFS	term (113 cm, 51 kg)	born alive	Multifocal hairless areas with keratosis-like thickening lateral and dorsal at neck, subcutaneous fibrous nodular mass at neck	Hyperextension of all fetlock joints, intracranial haemorrhage, fetal atelectasis. Heart beat present but no attempt to breathe	EAV neg. EHV1 neg. non-specific bacteria
13	Warmblood	WFFS/WFFS	10 months (120 cm, 48 kg)	born alive	Multiple skin defects on the neck, thorax, limbs and inguinal region	Stiff carpal and tarsal joints, abnormally flexible digital joints, multiple lesions of the aorta and V. cava, diaphragmatic hernia, extensive haemorrhage on left side of thorax	
14	Warmblood	WFFS/WFFS	term (126 cm)		Multiple skin lesions (both front legs, right hindleg, neck, flank and tail)	Stiff carpal joints, hyperextension of digital joints, severe scoliosis of thoracic spinal cord, brachygnathia superior, patent foramen ovale and ductus acteriosus, fetal atelectasis. Dystocia (Caesarean section)	
15	Warmblood	N/N	day 233 (73 cm)	born dead		Enlarged/swollen carpal and tarsal joints, retained placenta	
B. Foals submitted for routine diagnostic procedures without a specific suspicion for WFFS							
16	Warmblood	N/N	10 months (84 cm, 37 kg)	born dead			EHV1/4 neg.

(*continued on next page*)

Table 1 (*continued*)

No	Breed	WFFS genotype	Stage of pregnancy (crown-rump-length, weight)	Foal death	Skin lesions	Further lesions and findings	Microbiology
17	Dülmen Pony	N/N	10 months (24 kg)	born alive			
18	Trotter	N/N	11 months (105 cm, 66 kg)	born dead		Mineralisation of placenta	
19	unknown	N/WFFS	9 months (102 cm, 60 kg)	born dead		Necrotic foci in liver	EHV1 pos.
20	Warmblood	N/N	(50 kg)	born alive		Necrotic foci in liver, lung, thymus and spleen	EHV1 pos.
21	Warmblood	N/N	(56 cm, 9 kg)	born dead			EHV1/4 neg.
22	Thorough-bred	N/N	7 months (58 cm, 17 kg)	born dead		Hydronephrosis	EHV1/4 neg. non-specific bacteria
23	Shetland pony	N/N	4.5 months (25 cm, 1.1 kg)	born dead			
24	Thorough-bred	N/N	7 months (68 cm, 11 kg)	born dead			EHV1/4 neg. non-specific bacteria
25	Thorough-bred	N/N	8 months (58 cm, 11 kg)	born dead			EHV1/4 neg. non-specific bacteria
26	Warmblood	N/N	(49 cm, 6 kg)	born dead			
27	Thorough-bred	N/N	6 months (65 cm, 15 kg)	born dead		Mineralisation of placenta	EHV1/4 neg. non-specific bacteria
28	Warmblood	N/N	late abortion (94 cm, 34 kg)	born dead		Placentitis and vasculitis	EHV1/4 neg. Staph. dysgalactiae
29	Thorough-bred	N/N	8 months (75 cm, 25 kg)	born dead		Placentitis	EHV1/4 neg. Sc. equi ssp. zooepidem.
30	Warmblood	N/N	(53 cm, 5 kg)	born dead		Autolysis	
31	Warmblood	N/N	(72 cm, 15 kg)	born dead		Placentitis, hepatitis, pneumonia	EHV1/4 neg. non-specific bacteria
32	Warmblood	N/N	(45 cm, 6 kg)	born dead		Placentitis, pneumonia, myocarditis	Aspergillus spp.
33	Warmblood	N/N	9 months (90 cm, 27 kg)	born dead			EHV1/4 neg.
34	unknown	N/N	7 months (80 cm, 19 kg)	born dead			EHV1/4 neg.
35	Warmblood	N/N	(85 cm, 20 kg)	born dead		Necrotic foci in adrenal gland	EHV1/4 neg.
36	Thorough-bred	N/WFFS	10 months (100 cm, 58 kg)	born alive			EHV1/4 neg. non-specific bacteria
37	unknown	N/N	(80 cm, 19 kg)	born dead		Placental vasculopathy	
38	Warmblood	N/WFFS	8 months (72 cm, 14 kg)	born dead		Placentitis	
39	Warmblood	N/N	10 months	born alive		Placentitis	EHV1/4 neg. non-specific bacteria

(*continued on next page*)

Table 1 (*continued*)

No	Breed	WFFS genotype	Stage of pregnancy (crown-rump-length, weight)	Foal death	Skin lesions	Further lesions and findings	Microbiology
40	Warmblood	N/N	Late abortion (120 cm, 48 kg)	born dead		Necrotic foci in liver, thymus and spleen	EHV1 pos.
41	Warmblood	N/N	10 months (83 cm, 31 kg)	born dead		Hepatitis, splenitis, pneumonia with intranuclear eosinophilic inclusion bodies	
42	Thorough-bred	N/N	11 months (119 cm, 66 kg)	born dead			EHV1/4 neg. non-specific bacteria
43	Quarter horse	N/N	11 months (65 kg)				
44	Icelandic horse	N/N	Term (90 cm, 27 kg)	born alive, died on d 2			
45	Icelandic horse	N/N	Term (88 cm, 25 kg)	born alive, died on d 1			
46	Warmblood	N/N	11 months (104 cm, 53 kg)	Born dead		Necrotic foci in liver, adrenal medulla and thymus	
47	Warmblood	N/N					
48	Warmblood	N/N	day 260	born dead		No obvious external defects	EHV 1/4 neg.
49	Warmblood	N/N	day 220	born dead		No obvious external defects	EHV 1/4 neg.
50	Warmblood	N/N	day 183	born dead			EHV1/4 neg. *Actinobacillus equuli*
51	Warmblood	N/N	day 253	born dead		Retained placenta	
52	Warmblood	N/N	day 225	born dead		Posterior presentation, umbilical vessels congested, torsion of the umbilical cord	EHV 1/4 neg. no bacterial growth

Abbreviations: EHV Equine Herpesvirus, EAV Equine Arteritis Virus, pos. positive, neg. negative.

in 10% neutral buffered formalin, and routinely processed in paraffin wax. Tissue sections of 3 µm thickness were stained with haematoxylin and eosin, and evaluated microscopically.

3. Results

Of the 52 cases of abortion or stillbirth analysed in this study, 15 were submitted with a suspected diagnosis of WFFS (Table 1A) and 37 cases were presented for routine diagnostic procedures without any indication that WFFS was suspected as a cause for the abortion or stillbirth (Table 1B). Amongst the 15 cases for which there were indications the foetuses might be positive for WFSS, there were 14 homozygous carriers of the WFFS allele (WFFS/WFFS). There was detection of three foals that were heterozygous for WFFS (N/WFFS) in the group of cases submitted for routine diagnostic procedures. All other foals for which routine diagnostic procedures were conducted were WFFS negative (N/N). There was a presence of the c.2032A WFFS allele of the *PLOD1* gene in 15 Warmblood pregnancies (14 WFFS/WFFS, one N/WFFS), one Thoroughbred pregnancy (N/WFFS) and one pregnancy without a recording of the breed (N/WFFS; Table 1).

All pregnancies for which there were foetuses that were WFFS homozygous continued into very late stages of gestation and in most cases to a time similar to that when parturition would have normally occurred. Four foals were born in Month 9 ($n = 1$) and 10 ($n = 3$) of gestation and the other ten foals were born after a gestation length of at least 320 days (11 months; 10.8 ± 0.6 months). In contrast, abortions of foetuses negative or heterozygous for the WFFS allele occurred between 4 and 11 months of gestation (8.6 ± 1.9 months; $n = 27$, $P < 0.001$ compared with abortions of WFFS-homozygous foetuses), and there was no specific information on gestation length in mares with 11 WFFS non-homozygous foetuses.

The following description of clinical and patho-morphological findings refers only to the 14 pregnancies for which there were WFFS homozygous foetuses. Of the 14 foals, one was born dead, eight were born alive and five foals were dead at the time when observations of mares first occurred after parturition but no information was available on the status of these foetuses immediately after birth. Viability of the foals was impaired and reported information ranged from lack of breathing to spontaneous breathing and apparently normal reflexes. None of the foals ever attempted to stand. Foals born alive either died spontaneously ($n = 7$) or were euthanized ($n = 2$) for humane reasons at 2 min and 2 h after birth, respectively.

There were lesions or alterations of the skin of differing severities obvious in 12 foals (Figs. 1–4), in one foal these were restricted to the tail (Fig. 3). Histologically, there was a keratosis-like thickening of the skin and lateral or empty hair follicles in the affected areas were evident in one foal (Fig. 4) and besides skin defects there was a moderate hyperkeratosis on several skin locations in two other foals. In three foals, evaluations of histological sections indicated there were no major skin alterations (Table 2). Only in two foals, no skin lesions were detected (Fig. 5), but in one of these foals, the skin was abnormally thin. In one foal, there was a subcutaneous fibrous nodular mass in the neck area.

There was an abnormal flexibility of the digital joints in five foals (Fig. 3), flexed carpal joints in four foals and flexed tarsal joints in one foal. There also was an incomplete closure of the abdominal wall with prolapsed small intestines in one foal. In one foal, there were multiple perforating lesions of the aorta and V. cava and extensive intramuscular haemorrhage on the left side of the thorax. In addition to multiple skin defects, stiff carpal joints and abnormally flexible digital joints, there was a deformed spinal cord in two foals (Fig. 6). Pulmonary foetal lung dystelectasis but not a complete foetal atelectasis was evident in three of five foals submitted for necropsy while the other two foals had foetal atelectasis. There was an intracranial haemorrhage detected in two of four foals at the time of necropsy.

Five of the 14 foals required assistance at birth, including foetotomy for one foal and Caesarean section for another foal. Dystocia was caused by foetal malposition (dorso-ventral posture in one foal) and mal-posture (deviated head in one foal, flexed forelimbs in four foals). All mares with WFFS homozygous foals were Warmblood broodmares registered with a German breed registry. All these mares were bred to stallions that were heterozygous WFFS, however, this information was available after the pregnancy was confirmed. Mares had not been WFFS genotyped before breeding and this information was not available.

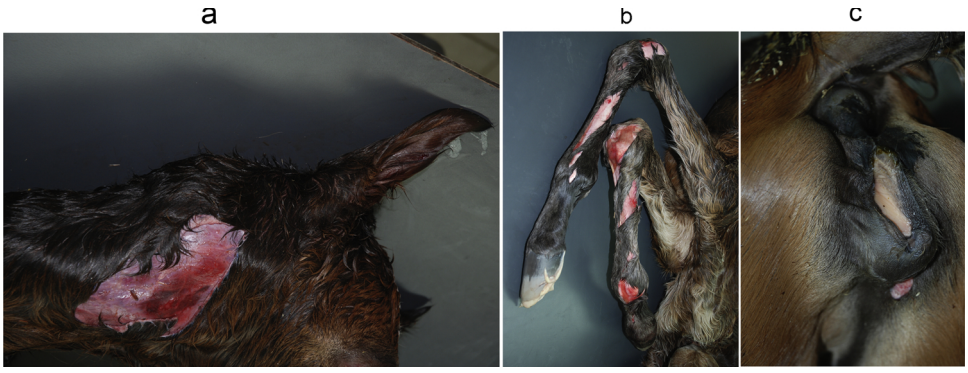

Fig. 1. Skin defects on the head (left), front legs (centre) and ventral to the vulva (right) in Foal 1.

Fig. 2. Superficial skin defects on the medial aspect of the right elbow in Foal 8.

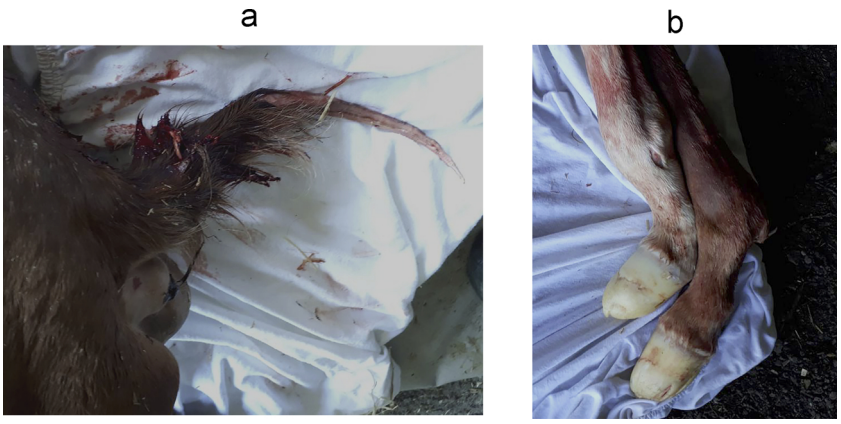

Fig. 3. Loss of skin on the distal part of the tail (left) and extremely flexible digital joints (right) in Foal 11.

4. Discussion

The results of this study indicate that foetuses of mares that are homozygous for the WFFS mutation of the *PLOD1* gene in most cases survive for the entire period of gestation. Foals are usually born alive but are non-viable. Abortions during the last months of gestation are not the predominant manifestation of the WFFS/WFFS genotype. There, therefore, is rejection of our initial hypothesis that most WFFS homozygous foetuses are aborted before the end of gestation, although WFFS-induced abnormalities of foetal development earlier in pregnancy cannot fully be excluded. Homozygosity of the WFFS allele is always incompatible with extra-uterine life.

Although there was not explicit exclusion of infectious causes of stillbirth and neonatal death in the present study, the conclusion that homozygosity for WFFS was the cause for abortion or stillbirth is highly likely. To the best of our knowledge, there are neither viable foals nor adult horses homozygous for WFFS (Müller-Herbst, unpublished). Thus, when there are horse foetuses that are WFFS homozygous, there is not birth of viable foals.

There were cutaneous defects detected in most but not in all WFFS homozygous positive foals in the present study. Cutaneous lesions were the predominant finding in a single WFSS case published previously (Monthoux et al., 2015). In the present study, the nature and extent of skin abnormalities varied considerably among foals and in one case, only the foal´s tail was affected. Hyperkeratosis in the affected areas occurred in some but not all foals. Skin lesions were not detectable in two foals at birth. The absence of any skin abnormalities, therefore, does not allow for discounting the WFFS homozygote condition as the primary factor for birth of stillborn foals. The existence of homozygous non-viable foals without obvious external lesions may also explain why there has not previously been WFFS diagnosed more frequently.

An incomplete closure of the abdominal wall as described previously (Monthoux et al., 2015) occurred in one WFSS positive foal in the present study. Further findings in the present study included abnormal flexibility of the digital joints and contracted forelimbs. Intracranial haemorrhage as detected in two out of four foals submitted to necropsy may be a further frequent finding in WFFS homozygous foals but requires investigating more cases.

With five WFSS positive foals requiring assistance at birth, the rate of dystocia in WFFS homozygous foals was greater than the reported 10% dystocia rate in the overall horse population (Ginther and Williams, 1996). Dystocia as a result of WFFS homozygous foals was caused by foetal malposture and malposition. Although the number of cases is too small for statistical analysis with regard to dystocia, it is suggested that with WFFS homozygous foetuses there tends to be a greater incidence of malposture of the foetus at birth.

The WFFS allele, either homozygous or heterozygous, almost exclusively exists in Warmblood horses. Only one of the pathological pregnancies from other breeds analysed occurred with a Thoroughbred pregnancy with a WFFS heterozygous genotype (N/WFFS).

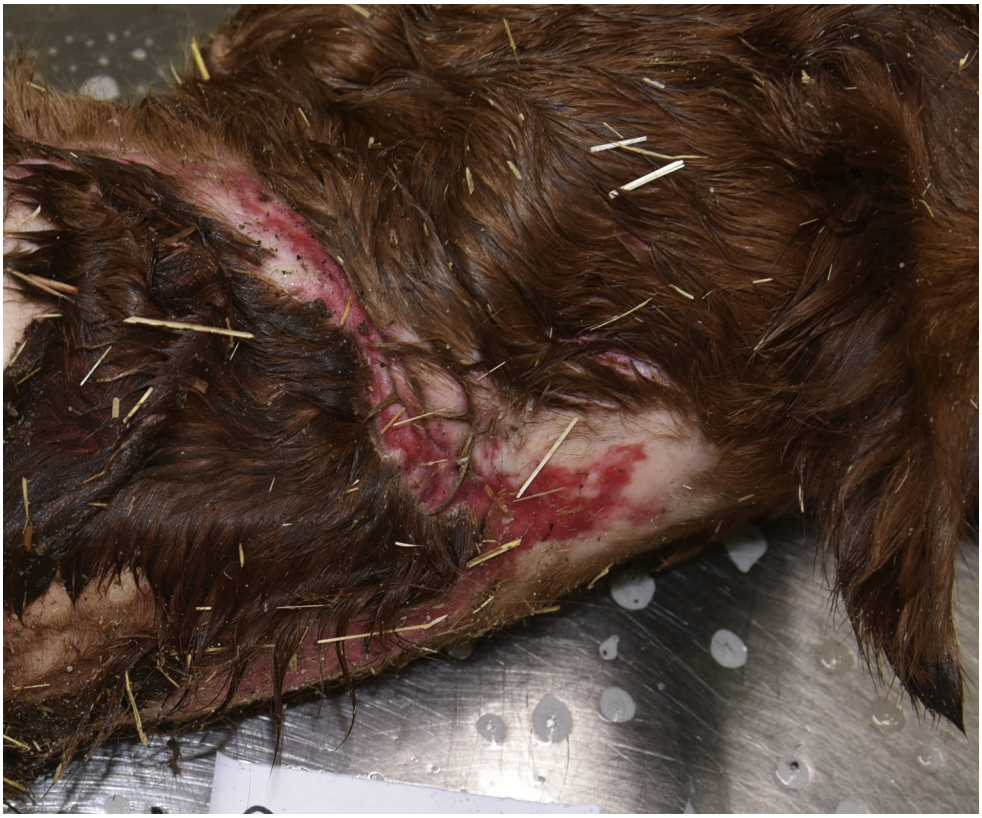

Fig. 4. Hairless areas with keratosis-like thickening of the skin lateral and dorsal at neck in Foal 12.

Table 2
Histological findings in foals homozygous for the WFFS Allele (WFFS/WFFS) submitted to necropsy (n = 5) or patho-histological assessment of skin samples (n = 1).

No	Skin	Further findings
1	Slight haemorrhage on skin lesions at neck, no further alterations at other skin lesions	Haemorrhage in cervical and pharyngeal muscles
3	Pigmented, regularly structured skin with normal hair and adnexal structures	(only skin sample submitted)
6	No pathological findings	Multifocal moderate inflammatory changes in brainstem
12	Proliferation of subcutaneous connective tissue, empty hair follicles, subepithelial haemorrhage	Foetal atelectasis, acute hyperaemia (lung, heart, kidney and brain)
13	Multifocal, moderate hyperkeratosis on several skin locations, moderate haemorrhage, perivascular inflammatory changes	Pulmonary dystelectasis, acute haemorrhage (lung, kidney, fatty tissue close to aorta and V. cava)
14	Moderate hyperkeratosis, dermal collagen fibres with reduced diameter and partially irregular alignment	Foetal atelectasis

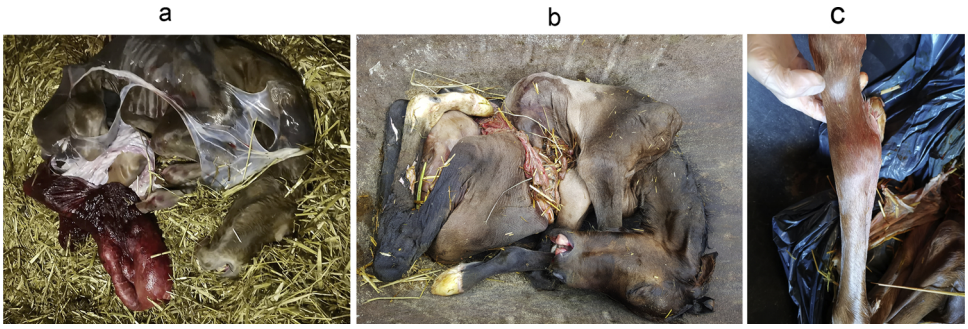

Fig. 5. WFFS homozygous foals with no apparent lesions (left: Foal 3, centre and right: Foal 7).

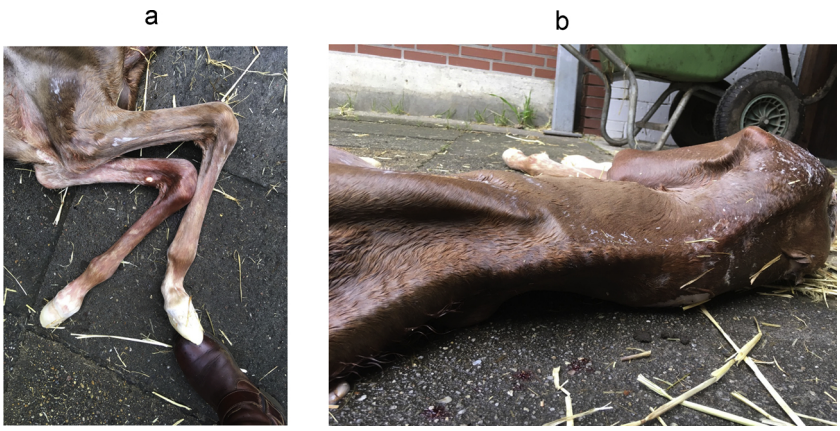

Fig. 6. Flexed forelimbs (left) and a deformed spinal cord (right) in Foal 4.

Only a limited number of aborted foetuses and foals from other breeds than the Warmblood were included in the present study. The aetiology of abortions and stillbirths in the non-WFFS-homozygous pregnancies of the present study represents a spectrum as encountered in many veterinary diagnostic institutions worldwide (e.g., Laugier et al., 2011) and includes Equine Herpesvirus 1, bacterial placentitis and non-infectious disease causes. None of these foals had skin lesions analogous to those of WFFS homozygous foals. A suspected diagnosis of the stillbirth of WFFS homozygous foetuses is justified, therefore, based on the clinical symptoms described in the present study.

Lesions comparable to those detected in the present study have been described in the 19th century in purebred Arab horses in Germany with close inbreeding with the Arab stallion, Bairactar, imported from Syria. Those foals were born alive near or at the end of a gestation period of normal length and had skin lesions primarily on the legs and back and occasionally, there were flexed forelimbs (Rueff, 1855). The pedigrees provided by Rueff (1855) are proof of an inherited condition. Bairactar can be found in the pedigrees of most if not all European Warmblood sport horses (Aurich and Köhler, unpublished). Although this does neither prove that Bairactar transmitted the WFFS allele nor that this stallion is the only source of WFFS in modern Warmblood horses, it may indicate that the condition did not develop in the Warmblood horse. The 19th century description does not allow for discounting other mutations leading to similar skin lesions (e.g., junctional epidermolysis bullosa - JEB). The JEB condition has been described in Belgian (Spirito et al., 2002), French (Milenkovic et al., 2003) and Italian draft horses (Capelli et al., 2015) and American Standardbreds (Lieto et al., 2002; Hierlmeier et al., 2013). These breeds are not among the ancestors of Warmblood sport horses whereas the Arab horse clearly is a part of the Warmblood ancestry.

The majority of WFFS homozygous foals in the present study had obvious morphological abnormalities. With the WFFS allele existing in horses potentially for at least two centuries, it remains unclear as to why the condition has not been previously described in more foals. With the genetic background of WFFS only discovered recently (Winand, 2011), such foals may have been classified as having malformations without suspecting a genetic predisposition for the conditions, therefore, there was no perceived need for further investigations. Assuming a 10% rate of presence of the WFFS allele in Warmblood mares and stallions (Moraes Dias et al., 2019) and random matings with regard to WFFS, statistically, one in 400 pregnancies is homozygous for the WFFS allele, corresponding to approximately 60 WFFS homozygous foals being produced per year in Germany. The cases in the present study were collected within a 5-month-period (January to May 2019), therefore, consisting of approximately two thirds of the foaling season. With initial information on WFFS provided to horse breeders in the spring 2018, it can be assumed that in that year matings were no longer totally at random with regard to WFFS. The number of 14 WFFS homozygous foals in the present study, therefore, includes at least 30% of all WFFS homozygous pregnancies in Germany during this time-period. To the best of our knowledge, this is a highly representative case collection for a genetic defect in horses that has not been investigated and described systematically.

In the present study, there was not assessment of the reason for foetal death with the WFFS homozygous pregnancies, but it is assumed that the foetuses died from multi-organ immaturity. Nevertheless, foetuses were apparently often able to initiate the endocrine cascade leading to parturition. It remains unclear why parturition in many cases was initiated slightly earlier than the average for Warmblood pregnancies. Pulmonary foetal lung dystelectasis but not complete foetal atelectasis was evident in two foals submitted for pathology, indicating that the foal was born alive but breathing did not proceed to normal inflation of the lungs. Pulmonary dystelectasis is not specific for WFFS homoyzygous foals but is a characteristic of foals born alive but that are unable to undergo normal neonatal adaptation to extra-uterine life.

Lesions due to WFFS appear to differ from those in hereditary equine regional dermal asthenia (HERDA). The HERDA is an autosomal recessively transmitted skin disease in Quarter horses that results in hyperextensible, fragile skin, seromas, haematomas and skin lesions in affected horses. Other than WFFS, HERDA is initially compatible with life and signs do not typically appear until horses are approximately 2 years old and there is starting of their equestrian training (Lerner and McCracken, 1978; White et al., 2004; Tryon et al., 2005; Litschauer et al., 2010).

In conclusion, birth of live but non-viable foals close to or at term is the predominant manifestation of WFFS in pregnancies. Abortions with a variety of less characteristic symptoms are rare. Economic losses in horse breeding caused by WFFS are thus greater

than previously assumed. Warmblood stallions, therefore, should be genotyped, a WFFS carrier status declared and there should not be breeding of WFFS carrier mares to carrier stallions.

Declaration of interest

The authors have no competing interests. SMH, EM and BG are affiliated with Laboklin. They were not aware of the clinical and pathological findings before submission of the manuscript.

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