

## Aborted fetal sizes of Thoroughbred horses in Hidaka, Japan, between 2005 and 2015

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*The degree of fetal growth restriction has been unclear in equine reproduction. In this study, 2,195 fetuses from 2,137 abortions during 11 seasons were examined to determine the causes of abortion, and fetal size dimensions (crown rump length and body weight) were measured. In total, 900 cases (42.1%) of abortion were identified as caused by viral infection (215, 10.1%), bacterial infection (156, 7.3%), fungal infection (25, 1.2%), circulation failure (406, 19.0%), multiple causes (66, 3.1%), deformity (13, 0.6%), placental abnormality (12, 0.6%), and other causes (7, 0.3%). All viral infections originated from equine herpes virus. Of all abortions, 94.3% occurred between 181–360 days of pregnancy, and the gestational ages at abortion were different based on the causes. Fetal sizes in viral abortions were considerably larger than those due to other reasons. Compared with viral infection, the crown rump length size dimension of fetuses aborted from multiple and fungal infection was affected. In addition, bacterial infection, circulation failure, and unknown causes of abortions also contributed to growth restriction in terms of body weight. In conclusion, the present study showed details of equine abortion and the relationships between causes of abortion and fetal size. Most of the aborted fetuses showed restrictions in their growth. The manifestations of growth restriction were more related to weight than skeletal length.*

**Key words:** abortion, causes of abortion, fetal size, FGR

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Equine aborted fetuses are often smaller than normal [1, 22]; however, there have been no extensive reports about aborted fetal size. Although the causes of equine abortion, stillbirth, and perinatal death have been reported in the U.S.A. [14, 15], U.K. [28], France [17], or Italy [18], the information about fetal size was not discussed in previous reports. Fetal growth restriction (FGR) in humans is induced by various factors and is associated with an increase in perinatal mortality and morbidity [7]. Similarly, in animal science, the available evidence suggests that FGR has permanent negative impacts on neonatal adjustment, preweaning survival, postnatal growth, feed utiliza-

tion efficiency, lifetime health, body composition, meat quality, and reproductive and athletic performance [32]. In pregnant women, prenatal care is regularly conducted, and fetal growth is monitored with ultrasonography [12]. FGR has also been reported in equine reproduction [26], and ultrasonographic fetal growth indices, such as the aortic diameter, biparietal diameter, transverse trunk diameter, or eye orbit, have been reported in normally [9, 20, 24, 25] and abnormally pregnant mares [1, 23].

Hokkaido Hidaka Livestock Hygiene Service Center is a public institution that has a role in examination and control of epidemics in Japan based on the Act on Domestic Animal Infectious Diseases Control. If mares abort in Hidaka, Japan, breeders transport the aborted fetus and placenta to the Livestock Hygiene Service Center, and a pathological appraisal is performed for investigation into the cause of abortion. Equine herpes virus infection is given special attention by breeders and government because of its virulence and classification as a notifiable infectious disease.

In the present study, we investigated the degree of FGR

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in equine abortions by comparing the causes of abortion, occurrence period, and aborted fetal size in Thoroughbred horses in Japan.

## Materials and Methods

A total of 2,605 aborted fetuses of Thoroughbred horses in 2,545 abortions were recorded at the Hokkaido Hidaka Livestock Hygiene Service Center over 11 seasons between 2004 and 2015. Of them, only data on cases of abortion (2,195 fetuses in 2,137 abortions), excluding stillbirth and neonatal death, were used.

Crown rump length (CRL) and body weight (BW) of aborted fetuses were measured. The causes of abortion were then examined based on bacteriological, virological, pathological, and serological examinations and categorized as infectious (virus, bacteria, and fungi), noninfectious (circulation failure, multiple pregnancy, deformity, placental abnormality, and other causes), and unknown. Circulation failure was diagnosed when there was excessive umbilical torsion or strangulation plus abnormalities of the circulatory system, such as umbilical edema or congestion, congestion or autodestruction of fetal organs, or presence of fetal hematoid ascites fluid. Deformity was defined as congenital anatomical malformation such as head deformity, abdominal wall closure insufficiency, or diaphragm aplasia. Placental abnormality was diagnosed macroscopically based on observations such as edema, thinning, or lack of villi.

### Bacteriological examinations

Bacterial species isolated from fetal tissues, stomach contents, or the placenta were cultured using 5% sheep blood agar and deoxycholate hydrogen sulfide lactose agar. Fungal culture and identification were also performed as required.

### Virological examinations

The presence of equine herpes virus was determined by the complement fixation reaction test or the loop-mediated isothermal amplification test [21] using fetal thymus or lung tissues.

### Pathological examinations

Tissue samples were collected from the placenta, mainly the cervical star portion, and some fetal tissues, such as the lung, heart, liver, spleen, kidney, and thymus. Adrenal gland, brain, or other tissues were also collected as needed. These tissues were fixed and imbedded in paraffin wax, and hematoxylin and eosin staining was performed. In addition, Gram staining (MacCallum-Goodpasture method), Periodic acid-Schiff staining, or immunostaining against *Aspergillus* or *Mucor* was performed as required.

### Serological examinations

Sera of aborted mares were collected, and agglutination tests for *Salmonella Abortusequi* [4] and an enzyme-linked immunosorbent assay and neutralization test for equine viral arteritis [27, 31] were performed for auxiliary diagnosis.

### Statistical analysis

Days of pregnancy at abortion were grouped into intervals of 20 days, and the CRL or BW data were analyzed by one-way analysis of variance followed by a Tukey's honest significant difference test for multiple comparisons in each period using JMP<sup>®</sup> 9 (SAS Institute Inc., Cary, NC, U.S.A.). Data are shown as means  $\pm$  standard deviation.

## Results

### Causes of abortion

Of the 2,137 abortions, the causes explaining the majority of cases were unknown ( $n=1,237$ , 57.9%), followed by noninfectious causes (504, 23.6%) and infectious causes (396, 18.5%) (Table 1). Of the infectious causes of abortion, abortions due to viral infection were the most frequent (215, 10.1%). Bacteria were the second most frequent cause of abortion (156, 7.3%), followed by fungal infection (25, 1.2%). All cases of viral infection were due to equine herpes virus type 1 (EHV1). *Streptococcus equi* subsp. *zooepidemicus* was most frequently isolated in abortions caused by bacteria (29.5%), followed by *Escherichia coli* (20.5%). *Salmonella Abortusequi* was detected in eight cases only during the 2007–2008 season. The major causes of fungal infections were *Mucor* sp. (48%) and *Aspergillus* sp. (24%). The noninfectious causes of abortion included circulation failure (406, 19.0%), multiple causes (66, 3.1%), deformity (13, 0.6%), placental abnormality (12, 0.6%), and other causes (7, 0.3%). Most multiple pregnancies involved twins, except for one case of triplets. Cases of deformity included six cases of diaphragm aplasia, six cases of head deformity, and one case of insufficient abdominal wall closure.

### Timing of abortion

Details regarding the timing of abortion are shown in Table 1. Of all abortions, 94.3% occurred between 181–360 days of pregnancy. The average and median ages of fetuses on the day of abortion are shown in Table 2.

### Aborted fetal size

Of the 2,195 aborted fetuses, useful CRL and BW data were available for 2,166 and 2,120 aborted fetuses (Fig. 1). The CRL and BW values of fetuses aborted due to viral infection were considerably larger than those of fetuses aborted due to other causes. Relative CRL and BW values compared with those of fetuses aborted due

**Table 1.** Diagnostic categories and gestational ages for 2,137 cases of equine abortion

Gestational age (days)	Infectious			Noninfectious					Unknown	Total (%)
	Virus	Bacteria	Fungi	Circulation failure	Multiple	Deformity	Placental abnormality	Other		
-100	0	0	0	0	0	0	0	0	6	6 (0.3)
101-120	0	0	0	1	0	0	0	0	4	5 (0.2)
121-140	0	2	0	2	0	0	0	0	15	19 (0.9)
141-160	0	8	0	7	0	0	0	0	11	26 (1.2)
161-180	0	8	0	12	3	0	0	0	34	57 (2.7)
181-200	0	14	2	44	3	0	0	0	86	149 (7.0)
201-220	3	12	1	76	7	0	2	0	139	240 (11.2)
221-240	3	21	2	74	10	0	1	0	114	225 (10.5)
241-260	19	24	0	56	13	1	0	0	102	215 (10.1)
261-280	39	18	6	73	11	1	2	0	128	278 (13.0)
281-300	53	20	10	41	6	4	2	0	146	282 (13.2)
301-320	63	9	3	13	8	1	4	1	139	241 (11.3)
321-340	31	17	1	5	4	4	0	3	203	268 (12.5)
341-360	4	3	0	2	1	2	0	3	103	118 (5.5)
361-	0	0	0	0	0	0	1	0	7	8 (0.4)
Total (%)	215 (10.1)	156 (7.3)	25 (1.2)	406 (19.0)	66 (3.1)	13 (0.6)	12 (0.6)	7 (0.3)	1,237 (57.9)	2,137

**Table 2.** Gestational days of abortion

	Infections			Noninfectious					Unknown
	Virus	Bacteria	Fungi	Circulation failure	Multiple	Deformity	Placental abnormality	Other	
Average	294.3	250.5	275.1	239.2	258.8	306.2	279.6	335.0	270.2
Median	298.0	251.5	287.0	236.0	258.0	301.0	287.0	338.0	278.0
SD	26.1	53.3	37.8	39.3	41.9	32.3	43.9	15.3	57.5

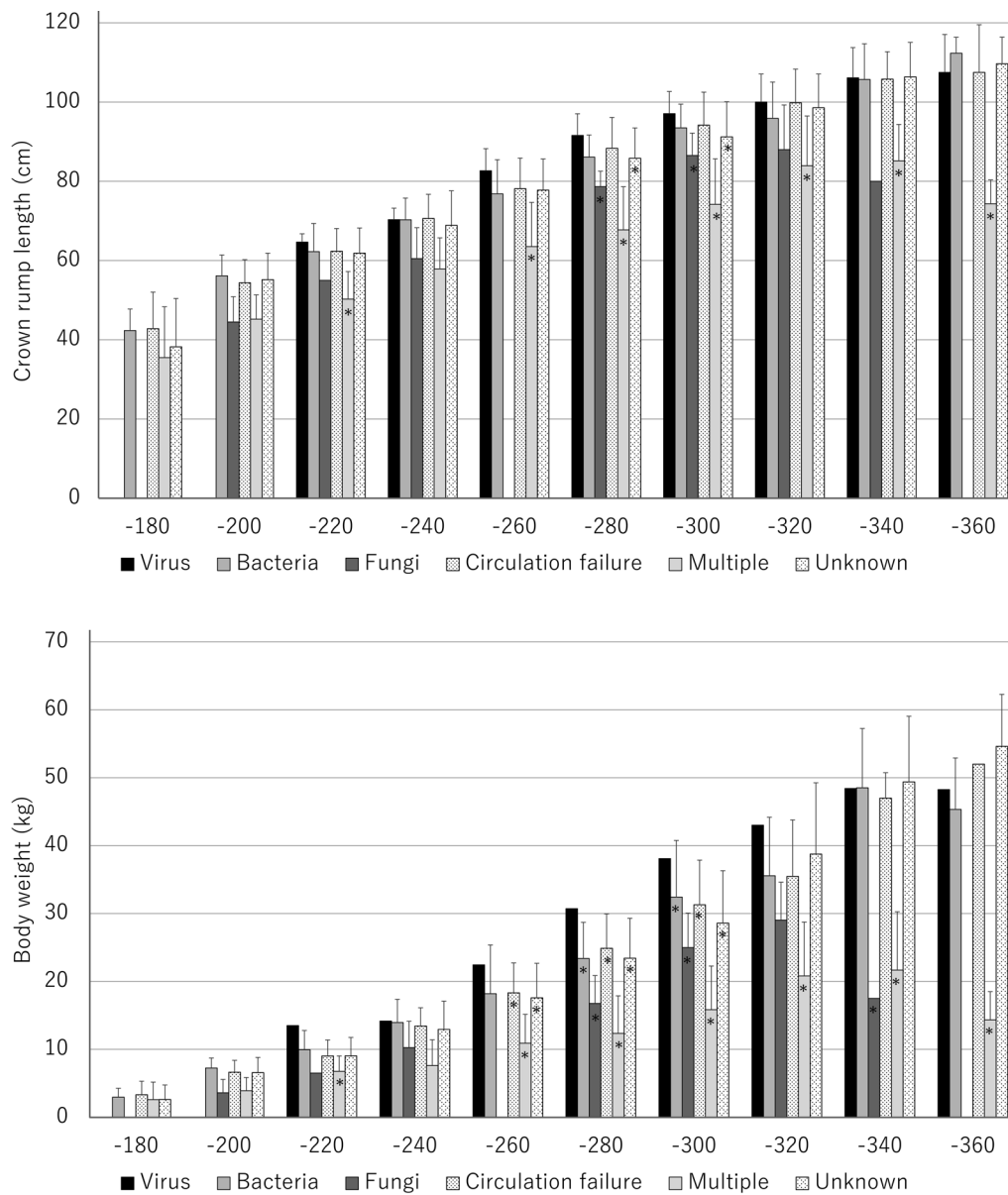
to viral infection are shown in Tables 3 and 4. Within the eight periods (201–360 days of gestational age) in which viral infectious abortion occurred, significant lower CRL values were measured in seven periods for multiple causes of abortion, two periods for fungal infection and unknown causes of abortion, and one period for deformity. Bacterial infection, circulation failure, and placental abnormality did not affect the CRL. On the other hand, significant lower BW values were measured over seven periods in abortions due to multiple causes; three periods in abortions due to fungal infection, circulation failure, and unknown causes; two periods in abortions due to bacterial infection; and one period in abortions due to deformity.

### Discussion

Our study summarized data concerning equine abortion in Hidaka, Japan. The growth of fetuses that aborted for certain reasons tended to be restricted.

Although some reports exist on equine abortion [2, 14, 15, 17, 18, 28, 29], there are few reports that focus on the timing of abortion [2]. The timing of abortion in the

present study differed because of the reasons given below. In infectious causes of abortion, viral infections are known to manifest during late pregnancy [30]. In the present study, 97% of viral abortions occurred after 240 days of pregnancy. On the other hand, bacterial abortions occurred during a wide range of days of pregnancy, and the range of days of pregnancy during which fungal abortions occurred was intermediate between those of abortions due to viruses and bacteria. Most twin fetuses were aborted from the 8th month of pregnancy to term [16], and 80.3% of twins were aborted after 221 days of pregnancy in the present study. Circulation failure and unknown causes were related to abortions beginning in the early period of pregnancy. About 58% of the causes of equine abortion were not identifiable in the present study. The reasons for this might be attributable to insufficient examinations, non-fresh samples, or without enough samples. We were unable to diagnose endocrine abnormality. Because of the characteristics of Hokkaido Hidaka Livestock Hygiene Service Center, it diagnoses infectious diseases in a strict manner, whereas the manner in which noninfectious diseases are diagnosed may be insufficient. In addition, unknown causes at term



**Fig. 1.** Comparisons of fetal size among causes of abortion. Body weight was more restricted than crown rump length. An asterisk (\*) indicates a significant reduction in comparison with viral abortion within a period. Placental abnormality, deformity, and other causes that explained less than 1% of total abortions were omitted.

may have included stillbirths by abnormal delivery, as we distinguished between abortion and stillbirth solely based on information provided by the breeders. Further studies are required to reveal the causes of equine abortion.

For evaluation of fetal restriction, a strict comparison with the normal fetal size was required. However, obtaining sufficient data for normal fetuses was difficult. Platt demonstrated that fetuses aborted as a result of infection with EHV-1 were of expected normal weight and size [22]. In our results, viral infection with EHV-1 occurred at 201–360 days

of pregnancy (eight periods), and fetal size was generally larger than in the case of fetuses affected by other factors. Thus, the sizes of fetuses aborted due to viral infection were considered normal in the multiple comparison test in the present study. The results showed that the values for CRL in 12 periods and BW in 19 periods in eight causes were significantly lower than those in viral infection during eight periods. In particular, many causes of abortion significantly affected both the CRL and BW between 241 and 300 days of pregnancy (three periods). In addition, relative BW

**Table 3.** Relative fetal crown rump length values for viral infection

Gestational age (days)	Infectious			Noninfectious					Unknown
	Virus	Bacteria	Fungi	Circulation failure	Multiple	Placental abnormality	Deformity	Other	
201–220	100.0	96.3	85.1	96.4	<b>77.7</b>	84.3			95.7
221–240	100.0	99.9	86.0	100.4	82.3	96.7			98.0
241–260	100.0	92.9		94.5	<b>76.8</b>		90.7		94.1
261–280	100.0	94.0	<b>85.9</b>	96.4	<b>74.0</b>	91.7	<b>54.6</b>		<b>93.7</b>
281–300	100.0	96.3	<b>89.1</b>	97.0	<b>76.4</b>	91.7	96.6		<b>94.0</b>
301–320	100.0	95.9	88.0	99.8	<b>83.9</b>	96.2	75.0	135.0	98.6
321–340	100.0	99.6	75.4	99.7	<b>80.2</b>		93.0	104.0	100.2
341–360	100.0	104.5		100.0	<b>69.1</b>		96.7	104.8	102.0

Values indicated in bold are significantly lower compared with viral infection by Tukey’s HSD test.

**Table 4.** Relative fetal body weight values for viral infection

Gestational age (days)	Infectious			Noninfectious					Unknown
	Virus	Bacteria	Fungi	Circulation failure	Multiple	Placental abnormality	Deformity	Other	
201–220	100.0	73.6	48.1	66.9	<b>50.0</b>	45.9			67.1
221–240	100.0	98.5	72.4	94.9	53.7	70.6			91.3
241–260	100.0	81.0		<b>81.6</b>	<b>48.6</b>		75.7		<b>78.3</b>
261–280	100.0	<b>76.1</b>	<b>54.5</b>	<b>81.0</b>	<b>40.2</b>	64.4	<b>42.3</b>		<b>76.2</b>
281–300	100.0	<b>85.1</b>	<b>65.6</b>	<b>82.2</b>	<b>41.6</b>	69.6	80.7		<b>75.1</b>
301–320	100.0	82.7	67.5	82.5	<b>48.4</b>	79.3	49.3	200.0	90.1
321–340	100.0	100.2	<b>36.1</b>	97.1	<b>44.8</b>		112.2	112.6	102.0
341–360	100.0	94.0		107.8	<b>29.7</b>		95.3	114.7	113.2

Values indicated in bold are significantly lower compared with viral infection by Tukey’s HSD test.

values tended to be lower than relative CRL values. For example, relative CRL and BW values were 94.0–104.5 and 73.6–100.2 for bacterial infection, 75.4–89.1 and 36.1–72.4 for fungal infection, 94.5–100.4 and 66.9–107.8 for circulation failure, 69.1–83.9 and 29.7–53.7 for multiple pregnancy, and 93.7–102.0 and 67.1–113.2 for unknown cases. These results suggested that some abnormal pregnancies might induce FGR. The statistical analysis conducted in the present study may have been inadequate because of the different numbers of samples during each period. In fact, the few significant differences before 240 days or after 301 days of pregnancy might have been the result of the small sample numbers used, whereas the range of 241–300 days of gestational age had large sample numbers and showed many significant differences.

Manifestations of FGR were categorized as symmetrical, in which an infant had a symmetrically smaller head and abdomen, or asymmetrical, in which an infant had a smaller abdominal size compared with its head size. Asymmetrical FGR is induced by undernutrition as a result of maternal, fetal, or placental abnormalities. The fact that most cases of abortion showed more restriction of BW than CRL might suggest that the fetuses were smaller and thinner than

normal fetuses. This is correspond to the fact that asymmetry is more frequent than symmetry in human [8].

Low birth weight (<90 lb, 40.8 kg) in foals is associated with twin pregnancy, fungal placentitis, other placental pathology, short gestation (<321 days), and FGR, although the birth weights of foals with bacterial infections are normal [22]. Our results showing that bacterial infection had less of an effect on FGR than fungal infection is in accordance with a previous report [22]. Bacterial infections which have been studied about examinations [5, 10, 11, 19] or treatments [6] as ascending placentitis did not significantly restrict CRL but did restrict BW. For evaluating fetal growth, soft tissue indices that can evaluate weight loss, such as the transverse trunk diameter, may be more useful than skeletal indices, such as the biparietal diameter or eye orbit.

Abortion due to unknown causes accounting for the majority of total abortions, also showed a tendency for FGR. This might suggest the usefulness of clinical monitoring of fetal size. However, it is difficult to directly link our results to usefulness in a clinical context because foal birth weight is affected by the parity of mares [13], size of mares [3], and normal fetal growth, particularly during late pregnancy. Thus, it is not clear to what degree FGR has been evaluated

by merely a single field examination.

In conclusion, our results showed details of equine abortion and the relationships between causes of abortion and fetal size. Most of the aborted fetuses including those that aborted due to unknown causes showed growth restriction. The manifestations of growth restriction were more related to weight than skeletal length.

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