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Potential Association Between Placental Features and Apgar Scores after Normal Parturition in the Thoroughbred Horse

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Abstract: Recently, the important role of the placenta in the well-being of the equine newborn has received more attention. It is well known that placenta efficiency mirrors the feto-maternal gas and nutritional exchanging. In the last years the APGAR score system has been proposed to assess the degree of peripartum asphyxia in foals. The aim of the present study was the assessment of potential association between placental features and APGAR scores after normal parturition in the Thoroughbred horse. Twenty-nine normal Thoroughbred foalings were observed and historical data concerning the mares were recorded. Each foal was assessed by the APGAR test within 20 min from birth and classified into one of the four classes of asphyxia. Before suckling each foal was weighed on an equine weight scale. Within 1 h of fetal membrane shedding, the placentae were classified grossly, depending on the presence of the lesions. Placenta samples were collected for histological examination. The results showed that a better APGAR class was positively correlated ($p<0.05$) to longer pregnancies and that the best APGAR class of foals was positively related to the placentae belonging to the best gross ($p<0.0001$) and histological class ($p<0.01$). A positive high correlation ($p<0.0001$) between gestational length and foal birthweight was also found, while no significant correlations between foal birthweight and both gross and histological classification were observed. In conclusion, the finding that foal APGAR class can be influenced by even mild abnormalities, underlines the importance of histological examination of placentae during the routine postpartum management of the mare and newborn.

Key words: Newborn foal, placenta, APGAR score

INTRODUCTION

It is well known that the placenta directly influences fetal development in mammals. During the seventies, several papers investigated gross and microscopic features of the equine placenta[1-5] and the characteristics of the normal term placenta of the horse were completely described by Whitwell and Jeffcott[6].

More recently, the important role of the placenta in the well-being of the equine fetus has received more attention, so that several authors reported studies on the aetiology, pathology, diagnosis, management and main effects of placental abnormality on the equine fetus and newborn[7-12].

An evaluation of placental structures and function can be performed during pregnancy by ultrasonography[12-14] or hormonal measurement, respectively[9,12,15-18].

As suggested by some researchers[12,19,20] evaluation of the equine placenta should be routinely performed after parturition. In fact, a thorough postpartum morphological assessment of the placenta provides valuable information on developmental diseases that could have affected the well-being of the fetus or could be a cause of weakness or disease in the newborn[12]. Cottrill *et al.*[21] evaluated the relationship between the foal outcome and the gross and histological examination of the placenta. These reserachers subdivided placentae into normal and abnormal, depending on gross abnormalities involving less or more than 10% of the total placenta area. Recently, Wilsher and Allen[22], using stereological methods, reported a study on the assessment of placental efficiency evaluated by foal birthweight as a function of total microscopic area of fetomaternal contact in Thoroughbred mares. The authors found that foal birthweight is a reflection of the balance between fetomaternal contact and placental efficiency.

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However the literature concerning the close relationship between viability of the newborn foal and placental features is still poor. For the human baby the Apgar test is used to evaluate the degree of peripartum asphyxia in the newborn and the need for resuscitation, by means of the evaluation of some parameters, such as heart rate, respiratory effort, muscle tone, reflex irritability and colour of mucous membranes[23,24]. A modified APGAR (Appearance, Pulse, Grimace, Activity, Respiration) score system has been proposed to assess the same parameters in foals[25].

The aim of the present study was the assessment of potential association between placental features and APGAR scores after normal parturition in the Thoroughbred horse.

MATERIALS AND METHODS

Mares: Twenty-nine normal foalings at a Thoroughbred stud were selected. All the mares were observed at parturition. All the foalings satisfied a set of criteria for normal parturition. The criteria adopted were necessarily arbitrary but were chosen in an attempt to eliminate parturition-related factors which might be a cause of newborn asphyxia. Many of the criteria were based on previous studies[6,19,26-28]. Foalings were only included in the study if:

- The mare delivered in recumbency
- Allantochorionic rupture and delivery did not require assistance
- The foal was in anterior dorsal presentation
- Second stage of labour (calculated from time of allantochorionic rupture until delivery of the fetus) took no longer than 20 min
- The umbilical cord was not cut or tied, but allowed to break naturally by movements of the foal or dam, after the establishment of respiration
- Placental expulsion took no longer than 2 h
- Historical data concerning the mares, such as age, parity and pregnancy length, were also recorded.

Foals: Each foal was assessed by the APGAR test within 20 minutes from birth. The foals were then classified into one of the four classes of asphyxia, as suggested in the modified APGAR scoring system[25]:

- APGAR class I: minimal asphyxia
- APGAR class II: mild asphyxia
- APGAR class III: moderate asphyxia
- APGAR class IV: severe asphyxia.

Before suckling each foal was weighed on an equine weight scale. The intervals of time from birth to stand up and to suck were also recorded.

Table 1: Mean (±SD) and actual range of maternal age, gestation length, foal birthweight, time to stand and time to suck of the foals, allantochorion area, allantochorion weight, umbilical cord length, in the 29 cases observed in the present study

Parameter	Mean±SD	Actual range
Mare age (y)	9.9±4.2	4-21
Gestational length (days)	337.9±11.4	320-355
Foal birthweight (kg)	41.9±5.6	35-55
Time to stand (min)	40±9	25-50
Time to suck (min)	74.8±13.9	40-90
Allantochorion weight (kg)	4.5±0.8	2.6-6
Allantochorion area (cm^2)	20641±3530.6	12061-27369
Umbilical cord length (cm)	48±7	36-73

Table 2: Distribution of the 29 Thoroughbred foals in APGAR classes

APGAR class I (minimal asphyxia) No (%)	APGAR class II (mild asphyxia) No (%)	APGAR class III (moderate asphyxia) No (%)	APGAR class IV (severe asphyxia) No (%)
18 (62)	9 (31)	2 (7)	0 (0)

Table 3: Distribution of the 29 placentae in Gross classes

Gross class 1 (absence of lesions) No (%)	Gross class 2 (lesions ≤10% of allantochorion-surface-area) No (%)	Gross class 3 (lesions >10% of allantochorion -surface-area) No (%)
19 (65.5)	5 (17.2)	5 (17.2)

Placentae:

Gross examination: Within 1 h of fetal membrane shedding, the placental integrity, the position and the extent of any gross lesions on the chorionic side were recorded. The surface area was measured as described by Whitwell and Jeffcott[6] and the allantochorion weight and the length of untwisted umbilical cord were recorded. The placentae were thus classified grossly, depending on the presence and the extent of the lesions as follows:

- Gross class 1: Absence of gross lesions
- Gross class 2: Gross lesions involving an area ≤ 10% of the total allantochorion surface
- Gross class 3: Gross lesions involving an area > 10% of the total allantochorion surface.

Histological examination: For enable valid comparisons of the placentae to be made, standardisation of the sampling was necessary. Samples were collected from the pregnant horn, non-pregnant horn, body and cervical star areas for histological examination. Any other areas that appeared grossly abnormal were also sectioned and submitted for histological examination.

The samples were fixed in 10% buffered formalin and embedded in paraffin wax. From each sample a 5 μm section was obtained and routinely stained with

Haematoxylin-Eosin. Microscopically, any pathological finding was recorded as:

- Absent (-)
- Mild (+/-)
- Moderate (+)
- Severe (++)

Density, dimension and appearance of placental villi were evaluated in 3 fields at low magnification (x 40)(LPF) in non-pregnant horn, pregnant horn and body sections only. The cervical star sections were not considered in this evaluation because of the normal absence of villi in this area. In each section a specific number of morphologically normal villi was considered as follows:

- Non-pregnant horn (NPH): normal(-) ≥ 3 villi/LPF; abnormal (+)<3 villi/LPF
- Pregnant horn (PH): normal(-) ≥ 4 villi/LPF; abnormal(+)<4 villi/LPF
- Body: normal(-)≥3 villi/LPF; abnormal(+)<3 villi/LPF

All the placentae were then classified into 2 groups as follows:

- Histological class A: placentae with all the sections characterized by the absence or the presence of mild pathological findings and, for the non-pregnant horn, pregnant horn and body sections only, by the presence of a normal number of morphologically normal villi in at least 2 sections.
- Histological class B: placentae with at least 1 section characterized by the presence of moderate or severe pathological findings and/or, for the non-pregnant horn, pregnant horn and body sections only, by the presence of a normal number of morphologically normal villi in less than 2 sections.

Statistical analysis: In order to assess any possible correlation between the APGAR classification and foal birthweight with respect to allantochorion weight, allantochorion area, umbilical cord length, gross classification and gestational length, the Kendall-tau non-parametric correlation test was used.

To evaluate any possible association between placental histological classification and foals APGAR classification the chi-square test was used. The influence played by the placental histological classification on foal's birthweight was assessed by using the biserial correlation test. The chi-square test was calculated with the MedCalc ver. 7.5.0.0. Biserial correlation represents the difference in two groups of measurements as a correlation coefficient, and can be calculated from mean, standard deviation and sample size for the two groups. Statistical significance was set at $p<0.05$.

RESULTS

Mares: The mean ±SD and the actual range of maternal age, gestational length, foal birthweight, time to stand and time to suck of the foals, allantochorion weight, allantochorion area and umbilical cord length, are reported in Table 1.

Foals: The results concerning the APGAR classification of the 29 Thoroughbred foals are shown in Table 2.

Placentae

Gross examination: The gross classification of the 29 placentae are shown in Table 3. Besides the well known small avillous areas at the tip of the pregnant horn, avillous areas covered by sticky green-brownish tanned material were also detected on the chorial surface in 10/29 (34.5%) placentae. The surface area of the lesions ranged between 1 to 7% in 5 placentae and between 13 to 15% in the remaining 5 placentae. These abnormalities involved the pregnant horn in 7 placentae, the body in 1 placenta, both horns in 1 placenta and both the body and the non-pregnant horn in 1 placenta.

Histological examination: The density of chorionic villi were different in different placental locations. The villi were particularly tall and wide in the non-pregnant horn and in the uterine body, small in the pregnant horn and absent in the cervical star. All the sections showed a cuboidal to columnar epithelium where bi- or multi-nucleated cells were sometimes detectable. Superficial and focal haemosiderin, hyperaemia and a mild oedema was always identified in the stroma. Macrophages and lymphocytes were occasionally detected. In the cervical star, raised linear avillous ridges, surrounded by short hypoplastic/atrophic villi, were always identified and a moderate oedema, hyperaemia, scattered haemorrhages and an increase of stromal cellularity were also detected.

The pathological findings observed, listed in Table 4, were always of minimal severity and distribution.

Table 4: Distribution of the pathological findings in placentae

Pathological findings	absent -	mild +-	moderate +	severe ++
Villous hypoplasia/atrophy	19		10	
Inflammatory cells	11	8	9	1
Haemorrhages	13	14	2	0
Squamous metaplasia	21	5	2	1
Fibrosis	20	6	3	0
Oedema	8	17	3	1

Table 5: Significant findings between foals' APGAR classification and birthweight, with respect to gestational length, and gross and histological placental classification

Variables	Kendall's Tau	Chi-square	p
APGAR class/ Gross classification	0.668		<0.0001
APGAR class/ Histological classification		9.886	<0.01
APGAR class/ Gestation length	0.270		<0.05
Foal birthweight/ Gestation length	0.604		<0.0001

According to the histological evaluation, 18/29 (62%) placentae were included in class A, while 11/29 (38%) were recorded in class B. The main pathological findings observed were villous hypoplasia/atrophy, infiltration of inflammatory cells (monocytes and neutrophils), squamous metaplasia, moderate haemorrhages, moderate fibrosis and oedema.

The typical abnormal areas detected at the tip of the pregnant horn were histologically characterized by atrophic villi with diffuse squamous metaplasia and often covered by a large amount of amorphous eosinophilic material.

The sections taken from gross lesions showed avillous areas with severe hyperkeratosis covered by abundant eosinophilic and amorphous material. A secondary stromal thickening, a stromal neo-vascularization and a villous and stromal infiltration of inflammatory cells (macrophages and neutrophils), were also present. No frank placentitis were seen.

The comparison between gross and histological placental classifications showed that overall 16 out of 19 (84.2%) placentas considered as normal at the gross examination, were histologically recorded in class A, while 3 (15.8%) were recorded in class B. Two out of 5 (40%) placentas which had gross lesions involving no more than 10% of the total allantochorion area, were histologically classified in class A and 3 (60%) in class B. All 5 (100%) of the placentas characterized by gross lesions involving more than 10% of the total allantochorion area, were histologically classified in class B.

Statistical analysis: The results of statistical analysis, limited to the significant findings, are shown in Table 5.

DISCUSSION AND CONCLUSIONS

In the last two decades a large number of researchers have analysed conditions of newborn foals and placental features, however their foal and placenta selection criteria varied considerably. Few authors have described the placenta from normal foaling[6,29], so that the reported data are often not comparable with the results of this study.

It is easy to understand how the foal birthweight mirrors the placental efficiency and the feto-maternal nutritional exchanges. The evaluation of this placental efficiency in the feto-maternal gas-exchange unit is more difficult. In this context, the APGAR scoring system modified by Vaala[25] was used because it represents a non-invasive, simple and useful method to evaluate the degree of asphyxia detectable in foals immediately after birth. The 62% of the foals were included in the APGAR class characterized by minimal asphyxia and none presented with severe asphyxia. Only two foals were recorded as moderately asphyxiated, but no pharmacological treatment was performed, and they spontaneously recovered soon after birth. It should be stressed that in the present study no severe newborn life-threatening condition occourred.

The birthweight recorded in the 29 Thoroughbred foals observed, ranged between 35 and 55 kg. This range is in agreement with that (36.8-63.2 kg) reported by Whitwell and Jeffcott[6].

The mean gestational length observed (337.9±11.4 days) is in agreement with that reported by Allen *et al.*[29] (338.3 ± 3 days) in Thoroughbreds.

The mean allantochorial weight recorded (4.5± 0.8 kg) is slightly higher compared with previously reported data (3-3.9 kg)[6,20,26,29,30]. Similarly, the mean allantochorial surface area (20641±3531 cm^2) is greater than the mean area (12900-16700 cm^2) measured by Rossdale[26], Whitwell and Jeffcott[6] and Allen[29].

The umbilical cord length, ranged between 36 and 73 cm, is in agreement with the range (32-90 cm) observed in normal foals by Whitwell and Jeffcott[6] and by Whitwell[31].

The frequent observation of avillous areas covered by sticky green-brownish tanned material at the tip of the pregnant horn is in agreement with findings reported by Whitwell and Jeffcott[6] and by Oikawa *et al.*[30]. suggested that these changes were a common fiding which accompany the progression of pregnancy. Although these changes were basically of a pathological nature, in accordance with Whitwell and Jeffcott[6], they were considered as non detrimental to the well-being of the foals.

The histological variety of villous morphology, the detection of haemosiderin and of focal haemorrhages is in agreement with data previously reported by Whitwell and Jeffcott[6].

The frequent finding of different degrees of hyperkeratosis observed in this study had been previously described by Cottrill *et al.*[21]. Those authors

hypothesized a relationship between this type of finding and foal limb deformities, not observed in the present study.

It is interesting to note that, in placentae demonstrating large gross lesions (>10%), mild histological changes were frequently detected also in sections taken from areas considered as grossly normal. In most of these sections mild villous hypoplasia/atrophy, multifocal hyperkeratosis, stromal neovascularization and inflammatory cell infiltration (macrophages and lymphocytes), were present. This could suggest that the grossly visible lesions were only the most obvious part of a general condition of placental abnormality. Moreover, mild histological abnormalities were detected in 16% of placentae grossly classified as normal. On the other hand, in the majority of grossly normal placentae, histology confirmed the absence of even minimal abnormalities.

The Kendall correlation test showed that a better APGAR class was positively correlated ($p<0.05$) to longer pregnancies, suggesting that gestational length could influence the grade of asphyxia in neonates.

The best APGAR class of foals appeared to be positively related to the placentae belonging to the best gross ($p<0.0001$, Kendall's Tau) and histological class ($p<0.01$, chi-square test). It is important to underline that these histo-pathological abnormalities were always very mild, however the cumulative action of mild abnormalities might have affected the APGAR classification of the foals.

A positive high correlation ($p<0.0001$) between gestational length and foal birthweight was found, which is in agreement with previous reported data[21,28].

No significant correlations between foal birthweight and both gross and histological classification were found. It seems then possible to argue that the presence of gross lesions involving no more than 15% of the allantochorion surface area, or the presence of mild histological abnormalities, did not significantly affect the degree of fetal body growth. On the other hand, they could affect the placental gas-exchange efficiency, as suggested by the influence exerted by both of them on the APGAR classification.

In conclusion, the finding that foal APGAR class can be influenced by even mild abnormalities, underlines the importance of histological examination of placentae during the routine postpartum management of the mare and newborn.

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