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Title: RELATION BETWEEN APGAR SCORING AND PHYSICAL PARAMETERS IN 44 NEWBORN AMIATA DONKEY FOALS AT BIRTH

Article Type: Original Research Article

Keywords: Donkey foal, Apgar score, neonatal evaluation, vitality

Corresponding Author: Dr. FRANCESCA BONELLI,

Corresponding Author's Institution: University of Pisa

First Author: FRANCESCA BONELLI

Order of Authors: FRANCESCA BONELLI; Irene Nocera, DVM; Giuseppe Conte; Duccio Panzani, DVM, PhD; Micaela Sgorbini, DVM, PhD

Abstract: The assessment of the behavior and physiological parameters of neonatal foals is essential in the detection of early signs of illness. Modified Apgar scoring systems from human medicine exist and have been validated in foals as a guide for assessing neonatal viability after birth. This study evaluated the viability of 44 Amiata donkey foals at birth, by assessing the Apgar score and comparing the relationship between viability and various physical parameters.
A total of 44 Amiata donkey foals and 27 jennies were enrolled in this study. An expert operator examined each foal within 5 minutes of birth. A complete physical examination was performed, along with an existing four-parameter Apgar score. The presence of the suckling reflex was evaluated. The interval time needed to acquire sternal recumbency and quadrupedal position, as well as nurse from the mare, were recorded. In addition, heart rate (HR), respiratory rate (RR), and rectal body temperature (BT) were measured. Results were expressed as median±standard error, minimum and maximum values.
The effects of the Apgar score on time to reach sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were estimated along with the differences related to Apgar scoring and gender. Differences between female and male donkey foals regarding the time to acquire sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were also assessed. Differences between female and male donkey foals regarding the Apgar score was evaluated using a chi-Square test. Finally, the reference values for Amiata donkeys were also calculated.
Twenty/44 (45.4%) foals were colts and 24/44 (54.5%) were fillies born from 27 jennies. None of the foals showed an Apgar score lower than 6. Twenty-nine out of 44 foals showed an Apgar score of 8/8, 10/44 a score of 7/8, while 5 foals (11.3%) showed a score of 6/8. No differences between fillies and colts in relation to the Apgar score were obtained.

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The authors confirm the following statements:

1. that there has been no duplicate publication or submission elsewhere of this work
2. that all authors have read and approved the manuscript, are aware of the submission for publication and agree to be listed as co-authors

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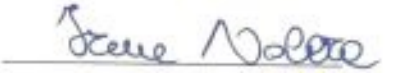
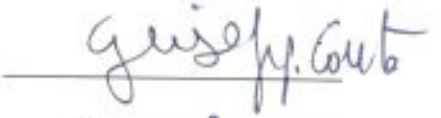
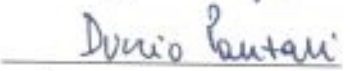
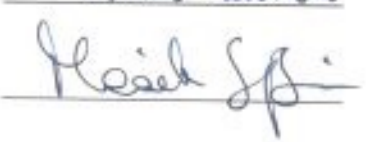
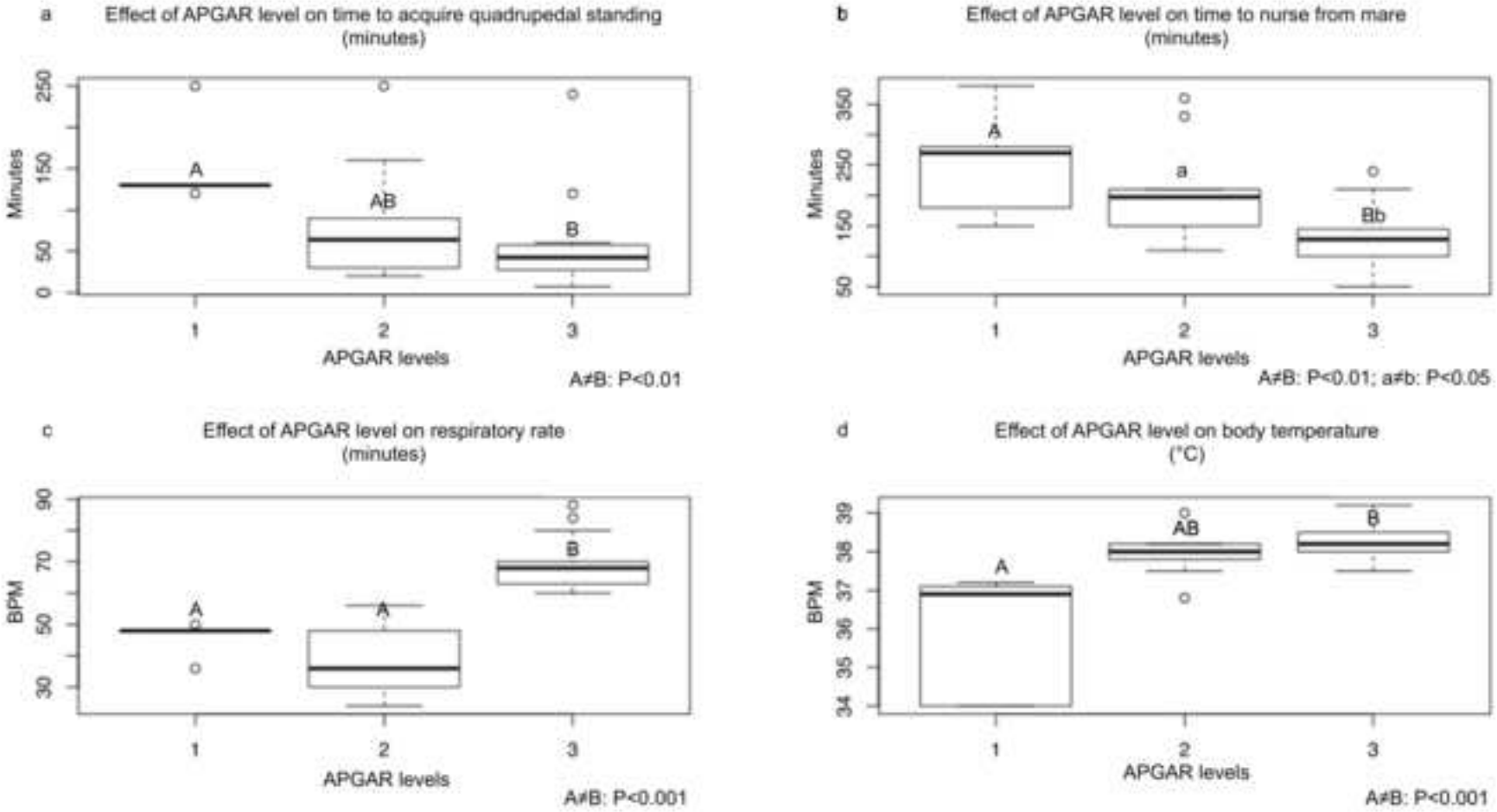
Francesca Bonelli	
Irene Nocera	
Giuseppe Conte	
Duccio Panzani	
Micaela Sgorbini	

Figure 1


a
Effect of APGAR level on time to acquire quadrupedal standing (minutes)
Minutes
0
50
150
250
A
AB
B
1
2
3
APGAR levels
A≠B: P<0.01
b
Effect of APGAR level on time to nurse from mare (minutes)
Minutes
50
150
250
350
A
a
Bb
1
2
3
APGAR levels
A≠B: P<0.01; a≠b: P<0.05
c
Effect of APGAR level on respiratory rate (minutes)
BPM
30
50
70
90
A
A
B
1
2
3
APGAR levels
A≠B: P<0.001
d
Effect of APGAR level on body temperature (°C)
BPM
34
35
36
37
38
39
A
AB
B
1
2
3
APGAR levels
A≠B: P<0.001

THERIO-D-19-00579 – REVISION 2

Dear Editor in chief for the Journal Theriogenology and dear Reviewers,

Thank for revising our manuscript and thank you for taking into consideration its publication in your journal. Please, find below the answers to your questions/concerns/comments.

Reviewer 1

1. Title - delete word "various" in the title. **The title has been changed according to the reviewer's comment (please see line 3).**
2. Highlights - Highlights 3 and 4 must to be rewritten according just the manuscript results.
3. Inclusion criteria of mares (data concerning age, parity, body weight (BW) and body condition score (BCS) were not showed and explained in the text. **The requested information has been added in the main text (Please see lines 146-148, 151-154, and 204-209).**
4. Number of animals per group (Apgar 6, 7 and 8) was very different to be used to calculate a "reference interval" for publication. **We agree with the Reviewer and in the original version of the manuscript we did not perform this kind of statistical analysis. However, one of the Reviewer strongly ask for adding it in the new version of the manuscript and we performed the analysis as he/she has been suggested. We will be fine with the final decision of the Editor. We have stressed more this limit in the main text (please see lines 242-243).**
5. Table 1 still out of the rules according guide of authors. **Changes have been made according to guide of authors.**
6. English writing has been little improved. **The reviewer has made comments about the level of English of our paper. In reality, this paper has been edited and proofread by Adrian Wallwork of English for Academics. Coincidentally, Wallwork also revises papers for some members of your department, he has also written a series of books for Springer Science on writing and presenting academic English. He has assured me that our paper is**

written in correct English. If you would like to contact him directly: adrian.wallwork@gmail.com.

e4ac
english for academics

11 August 2019

To whom it may concern

This is to declare that I have edited and proofread the English of the following paper:

RELATION BETWEEN APGAR SCORING AND VARIOUS PHYSICAL PARAMETERS IN 44 NEWBORN AMIATA DONKEY FOALS AT BIRTH

On behalf of:

Francesca Bonelli

My revision did not include the Bibliography. Subsequent to my revision, the authors may have made other changes or chosen not to implement some of the changes that I suggested. The correctness of the technical terms is also the responsibility of the authors.

The final version of the manuscript, as sent to the authors on **11 August 2019**, will be kept in our archives.

I have 30 years of experience of editing the English of scientific papers. I am also the author of *English for Writing Research Papers* published by Springer.

Please do not hesitate to contact me for any further information you may require:

adrian.wallwork@gmail.com

Best regards

Adrian Wallwork

English for Academics sas di Adrian John Wallwork
Via Carducci 9, 56127 Pisa Tel. 340 7888 304 (no fax) adrian.wallwork@gmail.com
p.iva / c.f. 01923950503 REA PI - 166038

Highlights

- The assessment of neonatal foal is mandatory to notice precocious signs of illness.
- The viability of Amiata donkey foals at birth was evaluated by the Apgar score.
- Physical parameters were compared, and the effect Apgar score on them was assessed.
- ~~The Apgar score was compared with some physical parameters.~~
- ~~The Apgar score was effective in assessing vitality in neonatal donkey foals.~~
- Apgar score relates to body temperature and respiratory rate, not to heart rate.

Research Article

RELATION BETWEEN APGAR SCORING AND PHYSICAL PARAMETERS IN 44 NEWBORN AMIATA DONKEY FOALS AT BIRTH

Francesca Bonelli[1], Irene Nocera[1], Giuseppe Conte[2], Duccio Panzani[1], Micaela Sgorbini[1]

[1]Department of Veterinary Sciences, University of Pisa, via Livornese snc, 56122 San Piero a Grado (PI), Italy. francesca.bonelli@unipi.it; irene.nocera@vet.unipi.it; duccio.panzani@unipi.it; micaela.sgorbini@unipi.it

[2]Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124, Pisa, Italy. giuseppe.conte@unipi.it.

Corresponding Author:

Dr Francesca Bonelli

Department of Veterinary Sciences

University of Pisa

Via Livornese snc – 56122 San Piero a Grado (PI), Italy

Tel. +390502210115

francesca.bonelli@unipi.it

Abstract

The assessment of the behavior and physiological parameters of neonatal foals is essential in the detection of early signs of illness. Modified Apgar scoring systems from human medicine exist and have been validated in foals as a guide for assessing neonatal

viability after birth. This study evaluated the viability of 44 Amiata donkey foals at birth, by assessing the Apgar score and comparing the relationship between viability and various physical parameters.

A total of 44 Amiata donkey foals and 27 jennies were enrolled in this study. An expert operator examined each foal within 5 minutes of birth. A complete physical examination was performed, along with an existing four-parameter Apgar score. The presence of the suckling reflex was evaluated. The interval time needed to acquire sternal recumbency and quadrupedal position, as well as nurse from the mare, were recorded. In addition, heart rate (HR), respiratory rate (RR), and rectal body temperature (BT) were measured. Results were expressed as median±standard error, minimum and maximum values.

The effects of the Apgar score on time to reach sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were estimated along with the differences related to Apgar scoring and gender. Differences between female and male donkey foals regarding the time to acquire sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were also assessed. Differences between female and male donkey foals regarding the Apgar score was evaluated using a chi-Square test. Finally, the reference values for Amiata donkeys were also calculated.

Twenty/44 (45.4%) foals were colts and 24/44 (54.5%) were fillies born from 27 jennies. None of the foals showed an Apgar score lower than 6. Twenty-nine out of 44 foals showed an Apgar score of 8/8, 10/44 a score of 7/8, while 5 foals (11.3%) showed a score of 6/8. No differences between fillies and colts in relation to the Apgar score were obtained.

Keywords

Donkey foal, Apgar score, neonatal evaluation, vitality.

1. Introduction

Donkeys (*Equus asinus*) have been close companions to humans for millennia and have been used as working animals all over the world [1]. Today, donkeys are also used in milk production for children with an intolerance to cow's milk [2,3] or in animal-assisted therapy [4]. The scientific literature has shown a renewed interest in these animals regarding their welfare [5], infectious diseases [6-8], alternative therapies [9], the need for specific diagnostic criteria and reference values in adult donkeys [10-13], pregnant and lactating jennies [14-16], and donkey foals [17-19].

Equine foals adapt rapidly to extra-uterine life, which is usually completed within a few days after birth. Normal foals are able to stand, to bond with the mare and show udder-seeking behaviour within a few hours of birth. A rapid adjustment of the foal's overall physiology to the extra-uterine life is crucial for their survival [20].

Peri-partum represents a very critical period. Data have shown that the mortality rate can reach 1.7% within the first 48 hours of a live birth [21]. Initial clinical signs of many systemic disorders may be vague and go unnoticed. For both practitioners and owners, it is therefore important to know the history of the mare (pregnancy and delivery), in order to evaluate the possible risk factors for perinatal diseases [22] and thus assess neonatal foal behavior very early on.

Ideally, the Apgar score should be performed within a few minutes of birth. Modified Apgar scoring systems from human medicine [23] have been developed and validated in foals as a guide for assessing neonatal viability [24-26].

Despite the large amount of literature on equine foals, there is a lack of knowledge regarding viability assessments of donkey foals at birth. The aim of the present study was thus to evaluate the viability of a cohort of 44 Amiata donkey foals at birth by assessing the Apgar score and comparing the relationship between the Apgar score and various physical parameters.

2. Materials and Methods

2.1 Animals

A total of 44 Amiata donkey foals and 27 jennies belonging to the Regional Stud Centre of Tuscany (Italy) were enrolled in this study. The Amiata donkey is one of the most common Italian donkey breeds originating from the Amiata mountain in central Italy. In 1990 the Biodiversity Committee of the European Parliament included the Amiata donkey in the list of endangered breeds (NL 215/90).

The donkey foals were born at the Veterinary Teaching Hospital “Mario Modenato”, Department of Veterinary Sciences, University of Pisa (n=27), at the Regional Stud Farm “Le Bandite di Scarlino” (Grosseto, Italy) (n=17) between 2005 and 2019.

All foals and jennies underwent similar management conditions. Jennies were housed in collective paddocks (8-10 animals/each) during pregnancy. Close to parturition (between 10 and 15 days), jennies were housed in individual 6x6 m boxes where after delivery they were kept with their foals until the second week post-partum. From sexual maturity, the jennies were trained to be separated from each other and spend some time in the box, however, due to their gregarious nature, the jennies were able to see and hear each other whilst in the box. Jennies were fed with meadow hay *ad libitum* along with commercial equine feed, in line with the NRC energy recommendations [27].

At inclusion time, data concerning age, parity, body weight (BW) and body condition score (BCS) [28] were recorded. Information regarding those jennies that had undergone more than one pregnancy and delivery throughout the study period, were assessed each time.

Approval to conduct this study was obtained from the Ethics Committee on Animal Experimentation of the University of Pisa and transmitted to the Italian Ministry of Health for the study-period 2005-2013 in line with the D.Lgs 116/92. Concerning the period between 2016 and 2019, the approval was obtained from the “Organismo Preposto al

Benessere Animale (OPBA), University of Pisa, according to the D.Lgs 26/14 (Prot. N. 33476/16).

2.2 Delivery management

The expected time for delivery was assessed by observation of mammary gland growth and calcium concentration in the milk. Mammary glands were examined visually every 24 hours and by palpation in order to assess the turgidity and filling degree [29,30]. When mammary secretion was present, the milk calcium concentration was evaluated every 24h at 6:00 pm using a commercial colorimetric kit (FoalWatch Titrets® for Daytime Foaling Management, Chemetrics, Inc., Calverton, VA USA). Since the electrolyte trend in jenny's milk near to parturition is similar to equine mares [31], an expert operator began to attend mares at night for parturition when the calcium concentration was >200 ppm, as indicated by the manufacturer. When the delivery started, the operator visually supervised each phase of the delivery. Care was taken to prevent stress throughout the procedures. In case of needs, the delivery was assisted by an expert operator.

2.3 Examination and sampling procedures

An expert operator examined each foal within 5 minutes of birth. A complete physical examination was performed, along with a four-parameter Apgar score, as proposed by others [25]. The presence of the suckling reflex was evaluated. The interval time needed to acquire sternal recumbency, quadrupedal position and nurse from the mare were recorded [30]. In addition, heart rate (HR), respiratory rate (RR), and rectal body temperature (BT) were measured. The naturally or manually detachment of the umbilical cord was recorded, along with the first spontaneous urination and meconium expulsion with or without the use of an osmotic enema. Foals were only restrained manually, and no drugs were used during the clinical examination.

2.4 Statistical Analysis

For statistical analysis, foals were retrospectively divided into three categories: 1) foals reaching an Apgar score of 8/8; 2) foals reaching an Apgar score of 7/8; and 3) foals reaching an Apgar score of 6/8. Results concerning physical parameters were evaluated for distribution using a Kolmogorov-Smirnov test. Data showed a non-Gaussian distribution and thus expressed as median±standard error, minimum, and maximum values.

The effect of the Apgar score on time to reach the sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were estimated by the Kruskal Wallis test and Dunn's multiple comparison test as a post-hoc analysis. Differences between female and male donkey foals regarding time to acquire sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were assessed using the Mann-Whitney test for unpaired data. Differences between female and male donkey foals regarding the Apgar score was assessed using a chi-Square test. Statistical significance was set at 0.05. Statistical analysis was performed using a commercial software (Graph Pad Prism, 6.0, USA).

Since no interval ranges of values are available for Amiata donkeys, lower and upper limit of reference interval were calculated using results of Apgar 7/8 and 8/8 foals by Reference Value Advisor software, as described by Geffrè et al. [32].

3. Results

Twenty/44 (45.4%) foals were colts and 24/44 (54.5%) were fillies, born during a fourteen-year study (2005-2019), from twenty-seven Jennies. Three out of 27 (11.1%) mares were primiparous. The median age was 6.9 years old (3-15 years), the median body weight was 346 kg (290-360 kg), and the median body condition score was 4/9 (3-7/9).

Two twin pregnancies were observed. Thirty-seven out of 42 (88%) deliveries were not assisted, while 5 out of 42 (12%) were assisted. No C-section were needed. Concerning the assisted deliveries, mares were all multiparous (second pregnancy in 2/5 jennies, third

154 pregnancy in 2/5 jennies and eighth pregnancy in 1/5 mare); 2/5 were twin pregnancies
155 (1/2 second pregnancy and 1/2 third pregnancy), while 3/5 were non-twin pregnancies (1/3
156 second and third pregnancy, respectively; 1/3 eighth pregnancy). None of the foals
157 showed an Apgar score lower than 6. Twenty-nine out of 44 foals (65.9%) (15/29 fillies
158 and 14/29 colts) reached an Apgar score of 8/8, 10/44 (22.7%) (8/10 fillies and 2/10 colts)
159 a score of 7/8, while 5 foals (11.3%) (4 males and 1 female) reached a score of 6/8; 2/5
160 were born from a twin pregnancy (1/2 was female, 1/2 male), and the related twins, both
161 males, showed an Apgar score of 7/8. Of the 5 donkey foals with an Apgar of 6/8, the 2/5
162 twins showed mild hypoxic-ischemic encephalopathy, 1/5 non-twins were septic and 2/5
163 non-twin foals showed signs of immaturity (pregnancy length >353±13 days). All the foals
164 that showed an Apgar score of 6/8 were born from an assisted delivery.
165 Suckling reflex was present in all the 44/44 (100%) foals within the physiological timing for
166 equine species (2-20 minutes) but was weak in 2/44 (4.5%) non-twin foals with an Apgar
167 score of 6/8, while strong in 42/44 (95.5%).
168 The umbilicus was naturally detached in 43/44 (97.7%) foals within physiological range
169 (15.9±5.2 minutes), while it was manually torn only in 1/44 (2.3%) foal with 8/8 Apgar
170 score. First urination was observed within the reference range for equine foals (by 8.5
171 hours) in all the donkey foals, meconium was naturally passed in 4/44 (9.1%) foals, while
172 an osmotic enema was administered after the first suckling in 41/44 animals (90.9%).
173 Table 1 reports the data concerning the median, minimum and maximum values for time to
174 reach sternal recumbency and quadrupedal position, time to nurse from the mare, HR, RR
175 and RT in relation to the Apgar values recorded.
176 Differences were found in terms of the time to acquire quadrupedal standing, to nurse the
177 mare, RR and BT among foals with different Apgar scores (6/8 vs 7/8 vs 8/8) (Figure 1),
178 while no difference was obtained for the time to reach sternal position (p=0.1) and HR
179 (p=0.31).

No differences were observed between female and male donkey foals for the time to reach sternal recumbency (p=0.565) and quadrupedal standing (p=0.402), to nurse from the mare (p=0.804), and on HR (p=0.302) and RR (p=0.687), while differences were observed between sexes and body temperature (p=0.033) values.

No differences were obtained between female and male donkey foals in relation to Apgar scoring (p=0.07).

The reference intervals for the time to acquire sternal recumbency, quadrupedal standing and to nurse, along with HR, RR and BT were reported in table 1.

4. Discussion

The Apgar scoring system was designed by Virginia Apgar in 1952 to provide a method to assess the newborn's condition at specific intervals after birth in humans in order to better assess the efficacy of resuscitation in newborn babies. The criteria for evaluation were skin color and appearance, pulse rate, reflex irritability, muscle tone and respiration [33]. Each criterion was determined on a scale from 0 to 2, with the sum of the five values resulting in an Apgar score that ranges from 0 to 10. In humans, the survival rate of newborns with an Apgar score <3 is usually considered as critical, from 4 to 6 is graded as less critical, and over 7 is regarded as normal [34].

The Apgar scoring system was introduced into veterinary medicine to assess the clinical status of foals [24-26], puppies [35,36] calves [37,38] and piglets [39,40] with variations compared to human newborn babies in order to assess the viability and effective perinatal asphyxia detection [23].

A modified Apgar score was developed for foals [24,25,41], consisting of evaluating the heart rate, respiratory rate, and muscle tone and irritability reflex. However, few data are available on the assessment of behavior and physical parameters in donkey foals at birth. In the present study, the Apgar score was assessed in a cohort of 44 donkey foals at birth.

All the jennies included in the present study did not show any risk factors which might have influenced pregnancy or increased foals' morbidity and mortality during the neonatal period. All the mares included could be considered "not old" (>15 years) (https://thehorse.com›breeding-the-older-mare), showed a BCS balanced for frame and covering [28] and a normal gestational time for this species [14]. Finally, no clinical signs of systemic or reproductive diseases were noticed during the all pregnancy-period.

In our study, the incidence of an Apgar score was equal to 6/8 due to a mild hypoxic-ischemic encephalopathy (2/44 foals, 4.5%) seems to be higher than in equine foals (1-2%) [42]. However, our population presented a relatively high prevalence of twins (4/44 foals, 9.1%), which might have been represented a bias. A high prevalence of poor viable donkey foals (2/5, 40%) were indeed composed of twins, which could explain the higher rate of hypoxic-ischemic encephalopathy found in our donkey foals. Usually, hypoxic-ischemic encephalopathy is associated with adverse peri-partum events, such as weak delivery, premature placental separation, and dystocia. In this study, 2/5 donkey foals with hypoxic-ischemic encephalopathy and an Apgar score of 6/8 were born after a twin pregnancy. In approximately 87% of twin pregnancies, mares abort or suffer dystocia, and twin foals suffer a higher rate of stillbirth and perinatal death [43].

The median values obtained in this cohort of donkey foals with normal viability regarding time to acquire sternal recumbency, heart and respiratory rates, and body temperature are similar to findings reported in equine foals in previous studies [26,44-46]. The time to stand in quadrupedal position was shorter compared to a previous study performed on Martina Franca donkey foals [30], while the time to nurse was longer [18,33,47]. However, both standing and suckling time were within the reference range for equine foals [26]. Establishing ranges for the time to stand and time to nurse might be a challenge for owners. Extensive knowledge of neonatal physiology is essential for recognizing the clinical abnormalities that are usually associated with systemic pathophysiological

changes. A better understanding of normal standing and nursing times might lead to a prompt identification of clinical signs related to any impairment during the neonatal period and to improve the animal's prognosis [48].

Analyzing our population of donkey foals in relation to the Apgar score assigned (8/8 or 7/8 or 6/8), the time to stand and to nurse were statistically shorter in foals with an Apgar of 8/8 compared to Apgar 7/8 or 6/8, while the time to reach sternal position was similar among the three groups. Immediately after parturition, cortisol levels are reported to be high in newborns in response to the stress of delivery, birth and adaptation to extra-uterine life [49,50]. A higher cortisol concentration might help even in foals with an Apgar score of 7-6/8 reach the sternal position. However, in the present study the number of foals with an Apgar score between 6/8 and 7/8 was lower compared to those with an Apgar score of 8/8. An increasing number of donkey foals with an Apgar score of less than 8/8 might be beneficial in order to improve the statistical analysis and lead to well-defined reference intervals that can help owners and practitioners dealing with a neonatal Amiata donkey foal.

The respiratory rate and body temperature were within the physiological equine foal range in donkey foals with a normal Apgar score (7/8 or 8/8), while foals with a low Apgar score (6/8), showed lower values [26]. In addition, both respiratory rate and body temperature were statistically lower in donkey foals with an Apgar score of 6/8 compared to those with normal Apgar values (7/8 or 8/8). The compromised respiratory rate in donkey foals with a low Apgar score might be related to abnormal breathing due to perinatal diseases (hypoxic-ischemic encephalopathy, immaturity/dysmaturity, septicemia). Sick foals may develop an abnormal low respiratory rate as a result of central nervous system damage or depression [24]. The significant decrease in body temperature in foals with an Apgar of 6/8 might be related to a decreased metabolic demand [51].

No differences were found among the three groups regarding heart rate, thus this parameter does not seem to be related to different Apgar scoring in donkeys. Foals with a low Apgar score and related neonatal pathology usually show an altered heart rate [24]. This difference might be due to the species, or to the relatively low number of animals included. An increased study population is needed in order to better understand the heart rate pattern in donkey foals with a low Apgar score (≤6/8).

In conclusion, the modified Apgar score assessed immediately after birth has proved to be an effective and easy method to assess vitality in neonatal donkey foals, also in field conditions. Further studies are needed to evaluate the value of the Apgar score in predicting the short-term survival rate of donkey foals. The interval ranges for clinical values of Amiata donkey foals at birth calculated in this study might be use as lower and upper limit of reference intervals for this breed. However, an increased study population would be needed for a deeper understanding of these results.

Figure Legend

Figure 1. Box-plot showing median, 1st and 2nd quartiles, minimum and maximum values for time to acquire quadrupedal standing (a), time to nurse from the mare (b) both expressed as minutes, respiratory rate expressed as breaths *per* minute (BPM) (c), and body temperature expressed as Celsius degree (d) in donkey foals grouped by Apgar score values. Legend: within graphs different letters denote a significant difference; °: outliers.

Authorship

FB – acquisition of data, drafting and revising the article, final approval of the version to be submitted; IN, DP and VV – acquisition of data; GC – analysis and interpretation of data. MS– design of the study, acquisition of data, interpretation of data, revising the article, final approval of the version to be submitted.

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	Apgar 8/8 (n=29)	Apgar 7/8 (n=10)	Apgar 6/8 (n=5)	Lower and upper limit of Reference interval
Sternal recumbency (minutes)	1±0.1 1-3	1.5±0.7 1-8	2±0.5 1-4	0.1 – 2.6
Standing time (minutes)	40±8.9 7-240	40±18.8 20-160	130±2.5 120-130	6.7 – 86.1
Time to nurse (minutes)	120±10.4 50-240	180±32.8 110-360	225±32.4 150-280	33.1 – 247.0
HR (bpm)	120±7.5 60-200	100±10.9 60-140	100±3.7 88-108	33.2 – 182.0
RR (bpm)	64±1.8 60-88	36±4.3 24-56	48±3.5 36-50	20.1 – 98.8
BT (°C)	38.3±0.1 37.5-39.2	38±0.2 36.8-39	36.9±0.7 34-37.2	37.1 – 39.2

Table 1. Data concerning the median±standard error, minimum and maximum values for time to acquire sternal recumbency and quadrupedal position, time to nurse from the mare, heart rate (HR), respiratory rate (RR) and body temperature (BT) in a population of 44 donkey foals with different Apgar scores. Lower and upper limit of reference interval for our population were also reported. Legend: bpm: beat per minute; bpm: breath per minute.

Research Article

RELATION BETWEEN APGAR SCORING AND ~~VARIOUS~~ PHYSICAL PARAMETERS IN 44 NEWBORN AMIATA DONKEY FOALS AT BIRTH

Francesca Bonelli[1], Irene Nocera[1], Giuseppe Conte[2], Duccio Panzani[1], Micaela Sgorbini[1]

[1]Department of Veterinary Sciences, University of Pisa, via Livornese snc, 56122 San Piero a Grado (PI), Italy. francesca.bonelli@unipi.it; irene.nocera@vet.unipi.it; duccio.panzani@unipi.it; micaela.sgorbini@unipi.it

[2]Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124, Pisa, Italy. giuseppe.conte@unipi.it.

Corresponding Author:

Dr Francesca Bonelli

Department of Veterinary Sciences

University of Pisa

Via Livornese snc – 56122 San Piero a Grado (PI), Italy

Tel. +390502210115

francesca.bonelli@unipi.it

Abstract

The assessment of the behavior and physiological parameters of neonatal foals is essential in the detection of early signs of illness. Modified Apgar scoring systems from human medicine exist and have been validated in foals as a guide for assessing neonatal

viability after birth. This study evaluated the viability of 44 Amiata donkey foals at birth, by assessing the Apgar score and comparing the relationship between viability and various physical parameters.

A total of 44 Amiata donkey foals and 27 jennies were enrolled in this study. An expert operator examined each foal within 5 minutes of birth. A complete physical examination was performed, along with an existing four-parameter Apgar score. The presence of the suckling reflex was evaluated. The interval time needed to acquire sternal recumbency and quadrupedal position, as well as nurse from the mare, were recorded. In addition, heart rate (HR), respiratory rate (RR), and rectal body temperature (BT) were measured. Results were expressed as median±standard error, minimum and maximum values.

The effects of the Apgar score on time to reach sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were estimated along with the differences related to Apgar scoring and gender. Differences between female and male donkey foals regarding the time to acquire sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were also assessed. Differences between female and male donkey foals regarding the Apgar score was evaluated using a chi-Square test. Finally, the reference values for Amiata donkeys were also calculated.

Twenty/44 (45.4%) foals were colts and 24/44 (54.5%) were fillies born from 27 jennies. None of the foals showed an Apgar score lower than 6. Twenty-nine out of 44 foals showed an Apgar score of 8/8, 10/44 a score of 7/8, while 5 foals (11.3%) showed a score of 6/8. No differences between fillies and colts in relation to the Apgar score were obtained.

Keywords

Donkey foal, Apgar score, neonatal evaluation, vitality.

1. Introduction

Donkeys (*Equus asinus*) have been close companions to humans for millennia and have been used as working animals all over the world [1]. Today, donkeys are also used in milk production for children with an intolerance to cow's milk [2,3] or in animal-assisted therapy [4]. The scientific literature has shown a renewed interest in these animals regarding their welfare [5], infectious diseases [6-8], alternative therapies [9], the need for specific diagnostic criteria and reference values in adult donkeys [10-13], pregnant and lactating jennies [14-16], and donkey foals [17-19].

Equine foals adapt rapidly to extra-uterine life, which is usually completed within a few days after birth. Normal foals are able to stand, to bond with the mare and show udder-seeking behaviour within a few hours of birth. A rapid adjustment of the foal's overall physiology to the extra-uterine life is crucial for their survival [20].

Peri-partum represents a very critical period. Data have shown that the mortality rate can reach 1.7% within the first 48 hours of a live birth [21]. Initial clinical signs of many systemic disorders may be vague and go unnoticed. For both practitioners and owners, it is therefore important to know the history of the mare (pregnancy and delivery), in order to evaluate the possible risk factors for perinatal diseases [22] and thus assess neonatal foal behavior very early on.

Ideally, the Apgar score should be performed within a few minutes of birth. Modified Apgar scoring systems from human medicine [23] have been developed and validated in foals as a guide for assessing neonatal viability [24-26].

Despite the large amount of literature on equine foals, there is a lack of knowledge regarding viability assessments of donkey foals at birth. The aim of the present study was thus to evaluate the viability of a cohort of 44 Amiata donkey foals at birth by assessing the Apgar score and comparing the relationship between the Apgar score and various physical parameters.

2. Materials and Methods

2.1 Animals

A total of 44 Amiata donkey foals and 27 jennies belonging to the Regional Stud Centre of Tuscany (Italy) were enrolled in this study. The Amiata donkey is one of the most common Italian donkey breeds originating from the Amiata mountain in central Italy. In 1990 the Biodiversity Committee of the European Parliament included the Amiata donkey in the list of endangered breeds (NL 215/90).

The donkey foals were born at the Veterinary Teaching Hospital “Mario Modenato”, Department of Veterinary Sciences, University of Pisa (n=27), at the Regional Stud Farm “Le Bandite di Scarlino” (Grosseto, Italy) (n=17) between 2005 and 2019.

All foals and jennies underwent similar management conditions. Jennies were housed in collective paddocks (8-10 animals/each) during pregnancy. Close to parturition (between 10 and 15 days), jennies were housed in individual 6x6 m boxes where after delivery they were kept with their foals until the second week post-partum. From sexual maturity, the jennies were trained to be separated from each other and spend some time in the box, however, due to their gregarious nature, the jennies were able to see and hear each other whilst in the box. Jennies were fed with meadow hay *ad libitum* along with commercial equine feed, in line with the NRC energy recommendations [27].

At inclusion time, data concerning age, parity, body weight (BW) and body condition score (BCS) [28] were recorded. Information regarding those jennies that had undergone more than one pregnancy and delivery throughout the study period, were assessed each time.

Approval to conduct this study was obtained from the Ethics Committee on Animal Experimentation of the University of Pisa and transmitted to the Italian Ministry of Health for the study-period 2005-2013 in line with the D.Lgs 116/92. Concerning the period between 2016 and 2019, the approval was obtained from the “Organismo Preposto al

Benessere Animale (OPBA), University of Pisa, according to the D.Lgs 26/14 (Prot. N. 33476/16).

2.2 Delivery management

The expected time for delivery was assessed by observation of mammary gland growth and calcium concentration in the milk. Mammary glands were examined visually every 24 hours and by palpation in order to assess the turgidity and filling degree [29,30]. When mammary secretion was present, the milk calcium concentration was evaluated every 24h at 6:00 pm using a commercial colorimetric kit (FoalWatch Titrets® for Daytime Foaling Management, Chemetrics, Inc., Calverton, VA USA). Since the electrolyte trend in jenny's milk near to parturition is similar to equine mares [31], an expert operator began to attend mares at night for parturition when the calcium concentration was >200 ppm, as indicated by the manufacturer. When the delivery started, the operator visually supervised each phase of the delivery. Care was taken to prevent stress throughout the procedures. In case of needs, the delivery was assisted by an expert operator.

2.3 Examination and sampling procedures

An expert operator examined each foal within 5 minutes of birth. A complete physical examination was performed, along with a four-parameter Apgar score, as proposed by others [25]. The presence of the suckling reflex was evaluated. The interval time needed to acquire sternal recumbency, quadrupedal position and nurse from the mare were recorded [30]. In addition, heart rate (HR), respiratory rate (RR), and rectal body temperature (BT) were measured. The naturally or manually detachment of the umbilical cord was recorded, along with the first spontaneous urination and meconium expulsion with or without the use of an osmotic enema. Foals were only restrained manually, and no drugs were used during the clinical examination.

2.4 Statistical Analysis

For statistical analysis, foals were retrospectively divided into three categories: 1) foals reaching an Apgar score of 8/8; 2) foals reaching an Apgar score of 7/8; and 3) foals reaching an Apgar score of 6/8. Results concerning physical parameters were evaluated for distribution using a Kolmogorov-Smirnov test. Data showed a non-Gaussian distribution and thus expressed as median±standard error, minimum, and maximum values.

The effect of the Apgar score on time to reach the sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were estimated by the Kruskal Wallis test and Dunn's multiple comparison test as a post-hoc analysis. Differences between female and male donkey foals regarding time to acquire sternal position and quadrupedal standing, time to nurse from the mare, RR, HR, and BT were assessed using the Mann-Whitney test for unpaired data. Differences between female and male donkey foals regarding the Apgar score was assessed using a chi-Square test. Statistical significance was set at 0.05. Statistical analysis was performed using a commercial software (Graph Pad Prism, 6.0, USA).

Since no interval ranges of values are available for Amiata donkeys, lower and upper limit of reference interval were calculated using results of Apgar 7/8 and 8/8 foals by Reference Value Advisor software, as described by Geffrè et al. [32].

3. Results

Twenty/44 (45.4%) foals were colts and 24/44 (54.5%) were fillies, born during a fourteen-year study (2005-2019), from twenty-seven Jennies. Three out of 27 (11.1%) mares were primiparous. The median age was 6.9 years old (3-15 years), the median body weight was 346 kg (290-360 kg), and the median body condition score was 4/9 (3-7/9).

Two twin pregnancies were observed. Thirty-seven out of 42 (88%) deliveries were not assisted, while 5 out of 42 (12%) were assisted. No C-section were needed. Concerning the assisted deliveries, mares were all multiparous (second pregnancy in 2/5 jennies, third

pregnancy in 2/5 jennies and eighth pregnancy in 1/5 mare); 2/5 were twin pregnancies (1/2 second pregnancy and 1/2 third pregnancy), while 3/5 were non-twin pregnancies (1/3 second and third pregnancy, respectively; 1/3 eighth pregnancy). None of the foals showed an Apgar score lower than 6. Twenty-nine out of 44 foals (65.9%) (15/29 fillies and 14/29 colts) reached an Apgar score of 8/8, 10/44 (22.7%) (8/10 fillies and 2/10 colts) a score of 7/8, while 5 foals (11.3%) (4 males and 1 female) reached a score of 6/8; 2/5 were born from a twin pregnancy (1/2 was female, 1/2 male), and the related twins, both males, showed an Apgar score of 7/8. Of the 5 donkey foals with an Apgar of 6/8, the 2/5 twins showed mild hypoxic-ischemic encephalopathy, 1/5 non-twins were septic and 2/5 non-twin foals showed signs of immaturity (pregnancy length >353±13 days). All the foals that showed an Apgar score of 6/8 were born from an assisted delivery.

Suckling reflex was present in all the 44/44 (100%) foals within the physiological timing for equine species (2-20 minutes) but was weak in 2/44 (4.5%) non-twin foals with an Apgar score of 6/8, while strong in 42/44 (95.5%).

The umbilicus was naturally detached in 43/44 (97.7%) foals within physiological range (15.9±5.2 minutes), while it was manually torn only in 1/44 (2.3%) foal with 8/8 Apgar score. First urination was observed within the reference range for equine foals (by 8.5 hours) in all the donkey foals, meconium was naturally passed in 4/44 (9.1%) foals, while an osmotic enema was administered after the first suckling in 41/44 animals (90.9%).

Table 1 reports the data concerning the median, minimum and maximum values for time to reach sternal recumbency and quadrupedal position, time to nurse from the mare, HR, RR and RT in relation to the Apgar values recorded.

Differences were found in terms of the time to acquire quadrupedal standing, to nurse the mare, RR and BT among foals with different Apgar scores (6/8 vs 7/8 vs 8/8) (Figure 1), while no difference was obtained for the time to reach sternal position ($p=0.1$) and HR ($p=0.31$).

No differences were observed between female and male donkey foals for the time to reach sternal recumbency (p=0.565) and quadrupedal standing (p=0.402), to nurse from the mare (p=0.804), and on HR (p=0.302) and RR (p=0.687), while differences were observed between sexes and body temperature (p=0.033) values.

No differences were obtained between female and male donkey foals in relation to Apgar scoring (p=0.07).

The reference intervals for the time to acquire sternal recumbency, quadrupedal standing and to nurse, along with HR, RR and BT were reported in table 1.

4. Discussion

The Apgar scoring system was designed by Virginia Apgar in 1952 to provide a method to assess the newborn's condition at specific intervals after birth in humans in order to better assess the efficacy of resuscitation in newborn babies. The criteria for evaluation were skin color and appearance, pulse rate, reflex irritability, muscle tone and respiration [33]. Each criterion was determined on a scale from 0 to 2, with the sum of the five values resulting in an Apgar score that ranges from 0 to 10. In humans, the survival rate of newborns with an Apgar score <3 is usually considered as critical, from 4 to 6 is graded as less critical, and over 7 is regarded as normal [34].

The Apgar scoring system was introduced into veterinary medicine to assess the clinical status of foals [24-26], puppies [35,36] calves [37,38] and piglets [39,40] with variations compared to human newborn babies in order to assess the viability and effective perinatal asphyxia detection [23].

A modified Apgar score was developed for foals [24,25,41], consisting of evaluating the heart rate, respiratory rate, and muscle tone and irritability reflex. However, few data are available on the assessment of behavior and physical parameters in donkey foals at birth. In the present study, the Apgar score was assessed in a cohort of 44 donkey foals at birth.

All the jennies included in the present study did not show any risk factors which might have influenced pregnancy or increased foals' morbidity and mortality during the neonatal period. All the mares included could be considered "not old" (>15 years) (https://thehorse.com›breeding-the-older-mare), showed a BCS balanced for frame and covering [28] and a normal gestational time for this species [14]. Finally, no clinical signs of systemic or reproductive diseases were noticed during the all pregnancy-period.

In our study, the incidence of an Apgar score was equal to 6/8 due to a mild hypoxic-ischemic encephalopathy (2/44 foals, 4.5%) seems to be higher than in equine foals (1-2%) [42]. However, our population presented a relatively high prevalence of twins (4/44 foals, 9.1%), which might have been represented a bias. A high prevalence of poor viable donkey foals (2/5, 40%) were indeed composed of twins, which could explain the higher rate of hypoxic-ischemic encephalopathy found in our donkey foals. Usually, hypoxic-ischemic encephalopathy is associated with adverse peri-partum events, such as weak delivery, premature placental separation, and dystocia. In this study, 2/5 donkey foals with hypoxic-ischemic encephalopathy and an Apgar score of 6/8 were born after a twin pregnancy. In approximately 87% of twin pregnancies, mares abort or suffer dystocia, and twin foals suffer a higher rate of stillbirth and perinatal death [43].

The median values obtained in this cohort of donkey foals with normal viability regarding time to acquire sternal recumbency, heart and respiratory rates, and body temperature are similar to findings reported in equine foals in previous studies [26,44-46]. The time to stand in quadrupedal position was shorter compared to a previous study performed on Martina Franca donkey foals [30], while the time to nurse was longer [18,33,47]. However, both standing and suckling time were within the reference range for equine foals [26]. Establishing ranges for the time to stand and time to nurse might be a challenge for owners. Extensive knowledge of neonatal physiology is essential for recognizing the clinical abnormalities that are usually associated with systemic pathophysiological

changes. A better understanding of normal standing and nursing times might lead to a prompt identification of clinical signs related to any impairment during the neonatal period and to improve the animal's prognosis [48].

Analyzing our population of donkey foals in relation to the Apgar score assigned (8/8 or 7/8 or 6/8), the time to stand and to nurse were statistically shorter in foals with an Apgar of 8/8 compared to Apgar 7/8 or 6/8, while the time to reach sternal position was similar among the three groups. Immediately after parturition, cortisol levels are reported to be high in newborns in response to the stress of delivery, birth and adaptation to extra-uterine life [49,50]. A higher cortisol concentration might help even in foals with an Apgar score of 7-6/8 reach the sternal position. However, in the present study the number of foals with an Apgar score between 6/8 and 7/8 was lower compared to those with an Apgar score of 8/8. An increasing number of donkey foals with an Apgar score of less than 8/8 might be beneficial in order to improve the statistical analysis and lead to well-defined reference intervals that can help owners and practitioners dealing with a neonatal Amiata donkey foal.

The respiratory rate and body temperature were within the physiological equine foal range in donkey foals with a normal Apgar score (7/8 or 8/8), while foals with a low Apgar score (6/8), showed lower values [26]. In addition, both respiratory rate and body temperature were statistically lower in donkey foals with an Apgar score of 6/8 compared to those with normal Apgar values (7/8 or 8/8). The compromised respiratory rate in donkey foals with a low Apgar score might be related to abnormal breathing due to perinatal diseases (hypoxic-ischemic encephalopathy, immaturity/dysmaturity, septicemia). Sick foals may develop an abnormal low respiratory rate as a result of central nervous system damage or depression [24]. The significant decrease in body temperature in foals with an Apgar of 6/8 might be related to a decreased metabolic demand [51].

No differences were found among the three groups regarding heart rate, thus this parameter does not seem to be related to different Apgar scoring in donkeys. Foals with a low Apgar score and related neonatal pathology usually show an altered heart rate [24]. This difference might be due to the species, or to the relatively low number of animals included. An increased study population is needed in order to better understand the heart rate pattern in donkey foals with a low Apgar score (≤6/8).

In conclusion, the modified Apgar score assessed immediately after birth has proved to be an effective and easy method to assess vitality in neonatal donkey foals, also in field conditions. Further studies are needed to evaluate the value of the Apgar score in predicting the short-term survival rate of donkey foals. The interval ranges for clinical values of Amiata donkey foals at birth calculated in this study might be use as lower and upper limit of reference intervals for this breed. However, an increased study population would be needed for a deeper understanding of these results.

	Apgar 8/8 (n=29)	Apgar 7/8 (n=10)	Apgar 6/8 (n=5)	Lower and upper limit of Reference interval
Sternal recumbency (minutes)	1±0.1 1-3	1.5±0.7 1-8	2±0.5 1-4	0.1 – 2.6
Standing time (minutes)	40±8.9 7-240	40±18.8 20-160	130±2.5 120-130	6.7 – 86.1
Time to nurse (minutes)	120±10.4 50-240	180±32.8 110-360	225±32.4 150-280	33.1 – 247.0
HR (bpm)	120±7.5 60-200	100±10.9 60-140	100±3.7 88-108	33.2 – 182.0
RR (bpm)	64±1.8 60-88	36±4.3 24-56	48±3.5 36-50	20.1 – 98.8
BT (°C)	38.3±0.1 37.5-39.2	38±0.2 36.8-39	36.9±0.7 34-37.2	37.1 – 39.2

Table 1. Data concerning the median±standard error, minimum and maximum values for time to acquire sternal recumbency and quadrupedal position, time to nurse from the mare, heart rate (HR), respiratory rate (RR) and body temperature (BT) in a population of 44 donkey foals with different Apgar scores. Lower and upper limit of reference interval for our population were also reported. Legend: bpm: beat per minute; bpm: breath per minute.

277 **Figure Legend**

278 Figure 1. Box-plot showing median, 1st and 2nd quartiles, minimum and maximum values
279 for time to acquire quadrupedal standing (a), time to nurse from the mare (b) both
280 expressed as minutes, respiratory rate expressed as breaths *per* minute (BPM) (c), and
281 body temperature expressed as Celsius degree (d) in donkey foals grouped by Apgar
282 score values. Legend: within graphs different letters denote a significant difference; °:
283 outliers.

Authorship

FB – acquisition of data, drafting and revising the article, final approval of the version to be submitted; IN, DP and VV – acquisition of data; GC – analysis and interpretation of data. MS– design of the study, acquisition of data, interpretation of data, revising the article, final approval of the version to be submitted.

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	Apgar 8/8 (n=29)	Apgar 7/8 (n=10)	Apgar 6/8 (n=5)	Lower and upper limit of Reference interval
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