Research article

Evaluation of jennies' colostrum: IgG concentrations and absorption in the donkey foals. A preliminary study

Luca Turini [a,b,c,*], Francesca Bonelli [a,b,c], Irene Nocera [a,b], Federica Battaglia [a,b], Valentina Meucci [a,b], Duccio Panzani [a,b,c], Marcello Mele [c], Micaela Sgorbini [a,b,c]

[a] Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, 56100, Pisa, Italy
[b] Ospedale Didattico Veterinario "Mario Modenato", Via Livornese snc, San Piero a Grado, PI, 56122, Italy
[c] Centro di Ricerche Agro-ambientali "E. Avanzi", University of Pisa, Via Vecchia di Marina, 6, 56122, San Piero a Grado, PI, Italy

ABSTRACT

Immunoglobulin type G (IgG) concentration both in jennies' colostrum and in serum of donkey foals are mostly unknown in the first 24 h after delivery. The aims of the present study were to evaluate the IgG concentrations of colostrum during the first 24 h of lactation of Amiata jennies, the absorption of colostrum and the weekly body weight gain of the donkey foals. IgG concentrations were assessed in the jennies' colostrum and in the serum of donkey foals. Colostrum was collected in 9 jennies ready after delivery, and at 6, 12, 24 h after foaling from both halves. Serum was collected at the same sampling times from 9 donkey foals. Donkey foals were weighted at birth and then weekly until the 28th days of life. Temporal changes of IgG concentrations in dam's colostrum and in donkey foal serum were analyzed by a linear regression model and a general linear model, respectively. Results showed that colostrum IgG concentration were similar between the left and the right half. Colostrum IgG concentrations decreased continuously throughout the time in all jennies by 0.0244 Log$_{10}$ mg/mL *per* hour. Serum IgG concentrations in donkey foals at birth was significantly lower compared to other times. No correlation was found between the colostrum IgG concentrations and the average weekly body weight gain of the donkey foal. The pattern of colostrum IgG levels in jennies and serum IgG concentration in donkey foals seem to be similar to what reported for equine. However, the donkey foals seem to be less agammaglobulinemic at birth compared to the horse foal. The pattern and both serum and colostrum concentrations evaluated in the Amiata donkeys were slightly different from results reported in other donkey breeds, underlying the importance of setting references specific to breed.

1. Introduction

Donkey (*Equus asinus*) has been used over the world mostly as a working animal [1]. Today, in the Mediterranean area, donkeys are mostly used for milk and meat production [2, 3], and in animal-assisted therapy [4].

Mammals placenta characteristics are broad and differ significantly between species. Despite a different number of chromosomes (donkey 62 *vs* horse 64), the placentation in donkeys and horses is similar. Donkey placenta is diffuse and epitheliochorial with numerous micro-placentomes consisting of a fetal microcotyledonary and a maternal microcaruncular part [5]. The characteristic of donkey placenta does not allow the transfer of a high quantity of immunoglobulins (Ig) from the dam to the foal throughout the intrauterine life. Due to the characteristic

of the placenta, which is similar to what reported for horses, it can be hypothesized that donkey foals born with a certain degree of immuno-globulins, but they need an adequate amount of a good quality colostrum in order to achieve the immunocompetence. Thus, the administration of dam's colostrum leads the foal to be protected from pathogens in the first months of life [6, 7].

Donkey colostrum is the first milk produced by the mammary gland after foaling and has a specific composition and a crucial role for the donkey foals. Colostrum has higher concentrations of Ig, anti-microbial and immunomodulatory factors, including lactoferrin, lactoperoxidase, lysozyme and oligosaccharides, and fat, compared to milk, along with a lower concentration of lactose [8, 9]. These characteristics give to the colostrum specific antimicrobial and anti-inflammatory properties. Despite the natural use of colostrum as first feeding for the foal, donkey

colostrum has been used in studies concerning the cryopreservation of the donkey semen, and for prevention of human atherosclerosis [10, 11].

The colostrum quality of the mare can be influenced by the age of the animal, health status, animal breed, lactation stage, length of previous lactation, management and environmental conditions, as well as feeding regimen [12, 13]. The main important risk factors for the failure of passive transfer (FPT) of immunity in the foal include feeding foal with a low quality of colostrum, the delay in suckling of the foal or the lack of absorption of immunoglobulins type G (IgG) [14, 15]. Mare colostrum is classified as very good if IgG concentrations are higher than 80 mg/mL, good between 50 and 80 mg/mL, fair between 28 and 50 mg/mL and poor when IgG concentrations are less than 28 mg/mL [16]. The healthy foal usually sucks colostrum from the dam within 2–3 h after birth [17]. In the first 12–24 h of life the small intestine remains permeable to macromolecules, including immunoglobulins. Approximately the 50% of ingested immunoglobulins are absorbed within the first 12 h after birth, while between the 12–18 h of life only the 28% of the ingested immunoglobulins are absorbed and passed into the bloodstream. After 18 h of life the efficiency of absorption continues to decrease until 36 h of life when the intestine is not anymore permeable [15]. The evaluation of FPT in the equine foal is assessed by the quantification of IgG serum concentration. Transfer of immunity from colostrum to the foal's circulation is considered adequate when serum IgG concentrations is >8 mg/mL, or as partial failure when IgG concentration is between 4 and 8 mg/mL. An IgG concentrations less than 4 mg/mL is considered a total failure of passive transfer [18]. Due to all these peculiarity of the peri-partum period in equids, is easy to understand why checking for colostrum quality and passive transfer of immunity might be so important.

The passive transfer of immunity in foals is important to protect the foal from diseases and it also contributes to maintaining a good growth rate in the first months of life. The relationship between growth rate and passive immune is well known underlining that healthy foals achieve a proper body weight gain compared to foals with FPT [7]. According to the Domestic Animal Diversity Information System (DAD-IS) [19], in Europe 60 breeds of donkey are recognized and almost for 28 breeds an accurate morphological description are reported [1]. Along with the breed characteristics, the donkey foal birth weight, and the average weekly body weight gain (AWG) could be very different. Thus, the investigation on both passive transfer of immunity and the average body weight and weight gain in the first weeks of life in a certain breed might represent a useful information for the farmer to establish the health status of the donkey foal [20].

The aim of the present study was to evaluate the IgG concentrations of colostrum during the first 24 h of lactation of the jennies, the absorption of colostrum and the AWG of the donkey foals.

2. Materials and methods

2.1. Animals

Ten Amiata jennies and their foals were included in this study. All the jennies belong to the Regional studfarm "Le Bandite di Scarlino" (Grosseto, Italy). The Ethical Committee of the University of Pisa (Organismo Preposto Benessere Animale, OPBA) approved the study with protocol number 22/19. An owner's written consent was obtained. All the jennies were housed at the Veterinary Teaching Hospital "Mario Modenato", Department of Veterinary Sciences, University of Pisa from the last two months of pregnancy until one months after the delivery in the 2019 foaling season.

All the jennies and their foals were equally managed. During the late pregnancy Jennies were housed in collective paddocks (3 animals/each), while they were house in individual boxes (6 × 6) 10–15 days before the presumptive delivery until 15 days after foaling. Foals were kept with their own dams. 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 grass hay ad libitum along with a commercial concentrate feed, according to the nutrient requirements stated by the NRC recommendations (National Research Council. Donkeys and other equids. Nutrient Requirements of Horses. Washington DC: The National Academy Press; 2007). Data concerning age, parity, body weight (BW) and body condition score (BCS) [21] were recorded.

2.2. Delivery management

In order to assess the expected time for foaling, both the mammary gland growth and calcium concentration of the mammary secretum has been checked. Mammary glands were examined visually every 24 h and by palpation for assessing the turgidity and filling degree [22]. As soon as Jennies started to produce mammary secretion, a commercial colorimetric kit (FoalWatch Titrets® for Daytime Foaling Management, Chemetrics, Inc., Calverton, VA USA) was used to evaluate the milk calcium concentration every 24 h at 6:00 pm. Since the electrolyte trend in jenny's mammary secretum near to parturition is similar to equine mares [23], 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. In case of needs, the delivery was assisted by an expert operator. Care was taken to prevent stress throughout the procedures.

2.3. Inclusion criteria

Donkey foals have been included in this study on the basis of the following criteria: (1) gestation length ≥372 days [24]; (2) unassisted delivery; (3) jennies treated against gastrointestinal parasites and vaccinated against equine influenza, tetanus, and equine herpes virus-1, according to guidelines of the American Association of Equine Practitioners Infectious Disease Committee (Adult Horse Vaccination Chart, 2010); (4) Apgar score ≥7,5 min after birth [25]; (5) presence of righting reflex immediately after foaling, suck reflex within 10 min, sternal recumbency within 5 min [26], quadrupedal position within 127.5 ± 70 min, and nursing the mare within 200 ± 67.4 min after birth [27]. Foals were physically examined before each weighting session, and they appeared to be clinically healthy during the study period. The APGAR score was evaluated 5 min after birth for each donkey foals. Only manual restriction was needed for all the procedures.

The behavior and the frequency of suckling were evaluated for each donkey foals during the first 24 h after foaling. Moreover, the jenny's mammary gland was evaluated by visually inspection and palpation after each meal.

2.4. Sampling and weighting procedures

Jennies' udder were cleaned by using soap and warm water in order to remove debris and bacteria [16]. The first strip of colostrum from each half have been throwed away. Then, ten mL of colostrum has been collected in a sterile milk tube at birth (T0-c), and at 6 (T6-c), 12 (T12-c), 24 (T24-c) hours after foaling from the left (l) and the right (r) half hand milked by a qualified operator and immediately stored at -20 °C until the evaluation. At the same sampling times reported for colostrum (T0-s, T6-s, T12-s, and T24-s), 10 mL of blood were collected from jugular vein of each donkey foals in order to assess the IgG concentrations. Samples were harvested in a red-top Vacutainer tubes (10-mL BD Vacutainer glass serum tube, silicone-coated; Becton Dickinson and Co., Franklin Lakes, NJ) and immediately centrifuged (Legend RT, Sorvall; ThermoFisher Scientific Inc., Waltham, MA) at 1.565 × g for 15 min in order to collect the serum. The serum samples were then stored at -20 °C until the evaluation.

Each donkey foal was firstly weighted immediately after the attending of the quadrupedal standing (T0-w), then they were weekly weighed until

the 28th days of life (T1-w, T2-w, T3-w and T4-w) with a dedicated scale (Wetoiml, Laumas® Elettronica, Italy). Briefly, both the jenny and the donkey foal were brought in the weighting area always by the same operators. One operator (FB) held the jenny close to the balance, while another operator (IN) put the donkey foal on the balance surface. A third operator (LT) recorded the data. The donkey foal was leave without any constriction until the result came on the balance screen (kg). Finally, both the jenny and the donkey foal were brought back in their box.

2.5. Single Radial Immunodiffusion (SRID)

Studies have been showed a strong homologue between donkey IgG and horse IgG [28, 29]. Because of the lack of a specific SRID for the donkey, and because of the close similarities of the immune systems of horses and donkeys, a SRID assay specific for horse was used to analyze IgG colostrum concentrations. All the colostrum samples were analyzed in a single batch. The colostrum pool (p) quality was calculated as the average quality of the 2 halves. SRID was performed using a Horse IgG IDRing (R) Test (IDBiotech, France). Test results were determined by comparison with a standard curve prepared using equine immunoglobulin standards supplied with the Kit. Colostrum and serum samples were diluted 1/600 and 1/150, respectively, before IgG determination because of their high IgG concentrations.

2.6. Statistical analysis

A Friedman test with a Dunn's test for multiple comparison was used in order to evaluate the differences between the colostrum quality for the left and right half and the colostrum pool at each sampling time. Since the test was not significant, the average value between left and right IgG measure was adopted in order to analyze the effect of time of sampling on IgG concentrations of jenny colostrum.

Data concerning colostrum IgG concentrations in jennies (as average of right and left half), serum IgG concentrations in donkey foals and foals' weights at different collection time were evaluated for distribution using a Shapiro-Wilk test. Since data about colostrum IgG concentrations showed a non-Gaussian distribution, a Log$_{10}$ transformation was applied in order to normalize the distribution. The results about colostrum IgG concentrations were thus analyzed with the following regression model and expressed as mean ± standard error.

$$Y_{ij} = m + b \, Time + Animal_j + e_{ij}$$

where: Y is the individual value of colostrum IgG per each jenny and time of sampling; Time is the continuous effect of time of sampling after the birth of the donkey foal, b is the regression coefficient; Animal is the random effect of jenny (j = 1 to 9); and e is the random error.

In order to evaluate the variation of serum IgG concentrations during the first 24 h after the birth of donkey foal, data of serum IgG concentrations were evaluated according to the following mixed model:

$$Y_{ijk} = m + Time_k + Animal_j + e_{ijk}$$

where: Y is the individual value of serum IgG concentrations per each donkey foal and time of sampling; Time is the fixed effect of time of sampling after the birth of the donkey foal (0h, 6h, 12h, 24h); Animal is the donkey foal random effect (j = 1 to 9); and e is the random error.

The AWG has been calculated. A Pearson correlation analysis was carried out in order to evaluate the correlation between the colostrum IgG concentrations and the AWG value.

Statistical significance was set at 0.05. A commercial software (Graph Pad Prism, 6.0, USA) was used for the statistical analysis.

3. Results

Nine out of ten jennies and their relative foal enrolled were included in the study. One foal, along with its own dam, was excluded because it died one week after birth. All the included jennies had a normal postpartum period and all the foals were healthy. The average parity number was 4.62 ± 2.34, while the average age of jennies was 12.11 ± 4.07 years. Four out of 9 (44.4%) donkey foal were male, while 5/9 (55.6%) were female.

All the donkey foals showed a proper suckling pattern, looking for drinking every 1 to 1 h and a half. All of them were able to empty both halves after each meal.

A total of 72 colostrum samples harvested from Jennies and a total of 36 serum samples collected from donkey foals at different sampling times were analyzed. Table 1 showed the results concerning the colostrum IgG concentrations (expressed as Log$_{10}$) evaluated by the SRID (Single Radial Immunodiffusion) analysis.

No statistically significant differences were found for IgG colostrum concentrations between the right and the left half. Thus, despite the analysis has been conducted considering both halves samples, we will refer to T0, T6, T12 and T24 instead of T0l/r, T6l/r, T12l/r, T24l/r. IgG colostrum concentrations at T0 was significantly higher compared to T6, T12 and T24 (p < 0.001), while at T6 it was significantly higher than at T12 and T24 (p < 0.001). There was no difference between IgG concentrations at T12 and at T24.

The mean ± standard deviation of IgG concentrations (expressed as Log$_{10}$) in the colostrum collected at different sampling times were reported in Table 2.

Colostrum IgG concentrations decreased continuously throughout the time in all jennies by 0,0244 Log$_{10}$ mg/mL *per* hour (Graph 1).

No correlation has been found between IgG colostrum concentrations of the jenny at T0 and IgG serum concentrations of the foal at T24. All the foals included in the present study reached a concentration of IgG in the serum >13 mg/mL.

IgG serum concentrations at T0 was significantly lower compared to T6, T12 and T24 (p < 0.001), while at T6 it was significantly lower than at T12 and T24 (p < 0.001). There was no difference between IgG serum

Table 1. Results on colostrum IgG concentrations (as Log$_{10}$) assessed in a population of 9 jennies for the right and the left half at different sampling times. The mean of the IgG colostrum concentrations of the two halves were also calculated as a "pool".

Animal	T0-r (mg/mL)	T0-l (mg/mL)	T0-p (mg/mL)	T6-r (mg/mL)	T6-l (mg/mL)	T6-p (mg/mL)	T12-r (mg/mL)	T12-l (mg/mL)	T12-p (mg/mL)	T24-r (mg/mL)	T24-l (mg/mL)	T24-p (mg/mL)
Jenny 1	1.92	1.92	1.92	1.89	1.95	1.92	1.59	1.89	1.76	1.41	1.81	1.65
Jenny 2	1.85	1.89	1.87	1.85	1.76	1.81	1.51	1.51	1.51	1.41	1.41	1.41
Jenny 3	1.92	1.92	1.92	1.76	1.80	1.78	1.51	1.51	1.51	1.41	1.41	1.41
Jenny 4	2.13	2.11	2.12	1.51	1.65	1.59	1.41	1.51	1.46	1.41	1.41	1.41
Jenny 5	1.89	1.89	1.89	1.59	1.41	1.51	1.51	1.51	1.51	1.41	1.41	1.41
Jenny 6	1.80	1.71	1.76	1.71	1.66	1.69	1.45	0.76	1.23	1.23	0.76	1.06
Jenny 7	2.11	2.15	2.13	2.09	2.06	2.08	1.51	1.98	1.81	1.51	1.41	1.46
Jenny 8	1.95	1.89	1.92	0.76	1.23	1.06	1.41	1.51	1.46	1.11	1.29	1.21
Jenny 9	1.89	1.89	1.89	1.81	1.76	1.79	1.59	1.51	1.55	1.41	1.29	1.35

T0 – after birth; T6 – 6 h after birth; T 12–12 h after birth; T24 – 24 h after birth; l – left half; r – right half; p – colostrum pool.

Table 2. Results concerning colostrum IgG concentrations (as Log_{10}), expressed in mean and standard deviation, evaluate in a population of 9 jennies for the colostrum pool at different collection time. IgG has been expressed as (mg/mL).

IgG mg/mL	T0	T6	T12	T24
Pool of colostrum	1.95 ± 1.42^a	1.76 ± 1.49^b	1.56 ± 1.17^c	1.40 ± 0.97^c

T0 – after birth; T6 – 6 h after birth; T 12–12 h after birth; T24 – 24 h after birth.
$a \neq b \neq c$ $p < 0.05$.

Table 3. Results on serum IgG concentrations assessed in a population of 9 donkey foals at different collection times.

	T0	T6	T12	T24
Serum IgG (mg/mL)	8.97 ± 0.50^a	11.91 ± 0.52^b	14.87 ± 0.5^c	14.91 ± 0.50^c

T0 – after birth; T6 – 6 h after birth; T 12–12 h after birth; T24 – 24 h after birth.
$a \neq b \neq c$ $p < 0.05$.

concentrations at T12 and at T24. The mean $\pm$ standard deviation of serum IgG concentrations were reported in Table 3.

The average weight of donkey foals at birth was 27.83 ± 5.40 kg. The AWG of the donkey foal was 0.841 ± 0.2 kg. No correlation was found between the colostrum IgG concentrations at different times and the AWG of the donkey foal.

4. Discussion

Despite the large number of studies present in literature regarding the passive transfer of immunity from mare to the equine foal, little is known for donkey foals. The evaluation of jenny colostrum could be useful to prevent and to assess the risk of FPT in donkey foals. Moreover, due to the largely variable in donkey breeds and sizes understanding the average concentrations of IgG in different breeds during the first hours of life may improve the neonate management. The aim of the present study was to evaluate the IgG of colostrum during the first 24 h of lactation of the Jennies, the absorption of colostrum in donkey foals and the weekly body weight gain of the donkey foal.

The quality of colostrum was considered as good or very good for all the jennies included in this study, according to McCue [16]. Literature found no differences between the right and left halves concerning the amount of milk produced [30], however there is a lack of information about the IgG concentrations between the two halves. According to the results of the present study, the IgG colostrum concentrations did not differ between the two halves. Colostrum IgG concentrations changed significantly throughout the different sampling times. The concentrations of IgG, in fact, was higher immediately after foaling (T0) and decreased significantly and continuously at T6, T12 and T24. These results agree with Erhard et al [14] who reported a reduction in the mean IgG concentrations in the post-partum mares from 54.5 mg/mL in the first 4 h after delivery to 10.1 mg/mL within 9–12 h postpartum. This trend could be due to the high production of IgG in the prenatal period and to the

physiologically transition between colostrum and milk that lead to a decreasing IgG concentration throughout the time [14, 31]. On the other hand, our findings were not in line with literature on jennies. In fact, Veronesi et al [32] found not statistically differences in IgG concentrations during the first 24h after parturition. However, the authors justified the lack of temporal changes to a possible wide interindividual variation in IgG concentrations at each sampling time and also to a possible different intraindividual profile. Considering the mean IgG concentrations, we found higher values in Amiata jennies than what reported for mares and Martina Franca jennies for all the sampling time [14, 32]. This might be due to interspecies and interbreed differences. Moreover, concerning the study of Veronesi et al [32], the authors started to sample the colostrum 5 days before the delivery. Milking the jennies before parturition, even collecting a small amount of colostrum, might have influenced the IgG concentrations found in the colostrum after foaling.

IgG serum concentrations in horse foals vary from 0.3 mg/mL in the pre-colostral phase to 15.7 mg/mL between 13 to 16 h after birth [14]. Results reported in the present study showed a higher amount of serum IgG in donkey foals at birth (8.97 mg/mL) compared to the equine foals. Other studies concerning birth evaluation of serum IgG concentrations in donkey foals seem to confirm that donkey foals showed higher serum IgG concentrations compared to equine foals in the pre-colostral phase [32, 33]. This difference between equine and donkey foals might be due to species related characteristics; however, to the best of our knowledge no studies compare the transfer of immunity during the fetal life in donkey and horses. Further studies might investigate those factors which could influence the transfer of immunity such as environment and feeding management.

In our population of Amiata donkey foals, serum IgG levels at birth were similar to findings reported in Pêga donkey [33], but higher compared to Veronesi et al (2014) [32] who investigated Martina Franca donkeys. These differences could be due to the different breed evaluated in the studies. Moreover, our results showed a statistically significant increasing in serum IgG concentrations from birth to 24 h of life, while

$$y = -0{,}0244x + 1{,}87$$
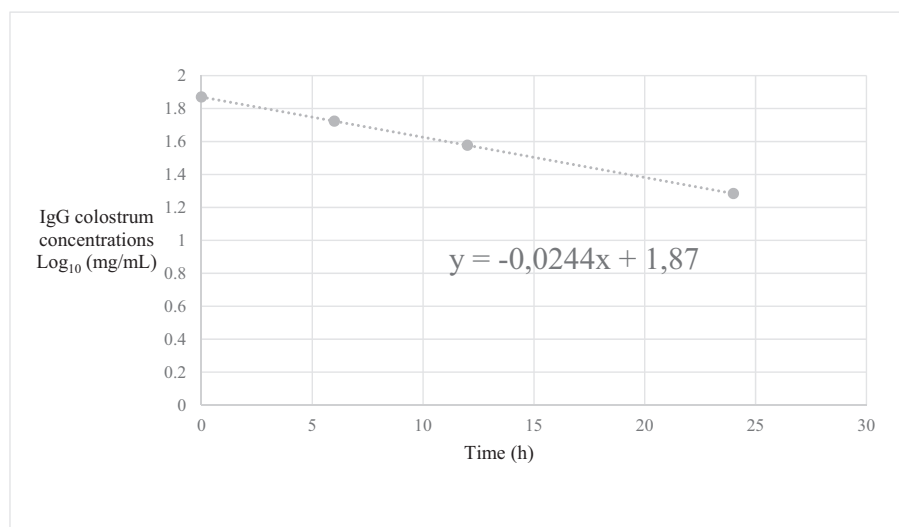
Graph 1. Colostrum IgG concentrations evaluated in a population of 9 Amiata jennies from delivery to 24 h after foaling. The IgG concentrations in the colostrum collected from the jennies were expressed as Log_{10}.

Veronesi et al [32] did not find significantly changes. These differences could be due to breed characteristic, but also to the pre-foaling colostrum. Milking the jennies 5 days before parturition might have influenced the IgG concentrations in the colostrum given to the donkey foals and the related amount immunoglobulins absorbed.

The colostrum IgG concentrations fed to the donkey foals were slightly different between each animal, however, all the subjects reached a concentrations of IgG in the serum >13 mg/mL at 24 h of life confirming that the cut-off for high quality colostrum suggested for mares might be suitable even for jennies [16]. The colostrum intake may have affected the serum IgG concentration of donkey foals and the lack of this information represents a limit of the present study. However, the suckling pattern of donkey foals was monitored through the first 24 h of life and a normal behavior was assessed for all the subjects [15]. Further studies evaluating the amount of colostrum intake of the donkey foal since birth to the 24 h of life might be useful in order to set a minimum volume of high quality colostrum required for reaching a complete transfer of immunity as reported in calves [34] and foals [7].

The relationship between growth rate and passive transfer of immunity can be important for breeders and owners leading to a better understanding of the donkey foals' health status. In this study, the average weight of donkey foals at birth and the AWG was lower compared to De Palo et al [20]. These differences might be due to breed related characteristic. Our results might represent a preliminary information concerning the average Amiata donkey foal birth weight and AWG. Moreover, our study showed no significant relationship between different colostrum IgG concentrations and the AWG of the donkey foals. This could be due to the lack of donkey foals affected by FPT in the study population [7]. This result confirmed what reported for calves fed by colostrum of different quality. Despite the difference in colostrum quality, no calves showed FPT and this was supposed to be the reason for the lack of significant relationship with the AWG [35]. Further studies might increase the number of animals included in order to set a range for the weight based on the breed and the gender.

Despite the high number of samples evaluated, a limit of the present study is the low number of animals included. Enroll a bigger population could give more strength to results.

5. Conclusion

In conclusion, the pattern of colostrum IgG levels in jennies and passive transfer of immunity in donkey foals seem to be similar to that reported for equine. However, the donkey foals seem to be less agammaglobulinemic at birth compared to the horse foal. Increasing the number of animals included could led to set reference ranges concerning colostrum quality and serum IgG concentrations in donkey foals specific for the Amiata breed.

Declarations

Author contribution statement

Luca Turini: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Francesca Bonelli: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Irene Nocera: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Federica Battaglia, Valentina Meucci: Contributed reagents, materials, analysis tools or data.

Duccio Panzani: Performed the experiments.

Marcello Mele: Analyzed and interpreted the data; Wrote the paper.

Micaela Sgorbini: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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