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Tiziana Nervo[a], Alessia Bertero[b,*], Gian Guido Donato[a], Elena Panier Suffat[b], Leila Vincenti[a].

[a] Dipartimento di Scienze Veterinarie, University of Torino, Torino, Italy;

[b] Practitioner, Dipartimento di Scienze Veterinarie, University of Torino, Torino, Italy.

[*] Corresponding author. E-mail address: alessia.bertero@gmail.com

Abstract

An inadequate colostrum intake results in Failure of Passive Transfer, a condition that makes foals more susceptible to potentially fatal infectious diseases. The aim of the study was to evaluate the transfer of passive immunity in the donkey, using electrophoresis as main diagnostic tool. A group of 20 Ragusana crossbreed jennies (age 3-19 years) and their foals were enrolled. The γ-globulin content of colostrum and dams' and foals' sera was measured, then the effects of foals' season of birth and age and parity of the jennies on γ-globulin concentration and on the efficiency of the immune transfer were evaluated. Influence of *season* factor was analysed by grouping the data on the basis of foaling season (spring, summer or autumn). For the evaluation of *age* and *parity* the jennies were divided into two categories: younger/older and primiparous/pluriparous, respectively. Finally, the possible association of these factors with the efficiency of the immune transfer was investigated. According to the horse reference range, 70% of donkey foals showed complete transfer of passive immunity (γ-globulin>8 g/L; 13.15±4.60 g/L) and 30% had a partial Failure of Passive Transfer (γ-globulin 4-8 g/L; 5.78±1.29 g/L), but without showing clinical signs. Age and parity did not significantly affect passive immunity transfer, nor did the season. Total Protein values measured through refractometer were positively correlated to the γ-

globulin content (r=0.69; p<0.01), confirming the possibility to use this diagnostic tool in the field as a first, inexpensive approach for colostrum evaluation.

Keywords: donkey, colostrum, passive immunity, age, season.

Highlights

1. The transfer of passive immunity in the donkey is still poorly investigated.
2. We investigated the transfer of passive immunity in donkeys using electrophoresis.
3. Influence of age, parity and season on the immune transfer has been evaluated.

1. Introduction

Although the global number of donkeys appears to be steadily decreasing (Bough 2011), the demand for donkey milk is increasing all over Europe due to its nutritional and cosmetic properties (Dai et al. 2019). The survival of each and every donkey foal is crucial for ensuring sufficient milk production, owing to the uniparity of the species and the long gestation period (372-374 days) (Wilborn and Pugh 2011; Carluccio et al. 2015).

After birth, the foal's immune system is immature and does not guarantee adequate protection, as it lacks circulating antibodies which do not cross the epitheliochorial placenta (Bernard and Barr 2012). The colostrum, taken during the first 24 h after birth, provides immunity to the foal for the first few weeks of life and its high protein content reflects the concentration of immunoglobulins (Ig) produced in late pregnancy. Of the four types of Ig (IgA, IgM, IgE and IgG), the IgG, represented by the γ-globulin family, are particularly important in foals (Perkins and Wagner 2015). In the horse, the intestinal absorption of Ig reaches its peak soon after birth, then it begins to drop, decreasing to 28% from 12 to 18 hours after birth (McKenzie 2018). This drastic fall is due to the replacement of specialized

enterocytes with mature ones, unable to absorb large proteins, which takes place regardless of whether or not colostrum intake has occurred and antibodies have been successfully absorbed (Bernard and Barr 2012). The first feeding is the one that provides the maximum Ig concentration, which is destined to rapidly decrease (Brinsko et al. 2010). As a consequence, the foal should receive colostrum within the first 2 hours after birth (Jeffcott 1972; Vivrette 2011). Within 4-6 hours after ingestion, Ig enter the foal's bloodstream, peak 24-48 hours post parturition and subsequently decline due to protein catabolism and plasma volume expansion (Bernard and Barr 2012). Initially, serum Ig concentrations in the foal reflect the maternal ones, then progressively decrease as a result of their utilization (Perkins and Wagner 2015).

The process of intestinal absorption and the use of colostral antibodies by the foal is called 'transfer of passive immunity' and any obstacle results in an inadequate concentration of serum Ig in the newborn, called 'Failure of Passive Transfer' (FPT) (Jeffcott 1972). A close relationship has been demonstrated between low serum IgG concentration in foals and the incidence of neonatal diseases (Vivrette 2011). In the horse foal, FPT is a widely studied condition associated with IgG serum concentrations under 4 g/L, 12 hours after birth, while regarding the donkey, the knowledge is currently very limited (Veronesi et al. 2014; Turini et al. 2020a, b) and, at present, no rapid tests are available for the specific measurement of Ig in this species.

Even though Radioimmunoassay is the gold standard for quantifying serum Ig (IgG-RID), semi-quantitative field tests are the most widely used as rapid diagnostic methods in horses (Lester 2011; Vivrette 2011; Kummer et al. 2018; McKenzie 2018). A positive correlation has been demonstrated between the IgG measured by electrophoresis (EGG, Electrophoretic Gamma Globulins) and the IgG-RID. Thus, the use of electrophoresis to predict FPT has been tested to demonstrate its field suitability (Tscheschlok et al. 2017).

This study is aimed to investigate the transfer of passive immunity in the donkey foal, using electrophoresis as the main diagnostic tool to evaluate the IgG in serum and colostrum. Furthermore, the possible influence of age and parity of the jennies and season of the year on the immune transfer has also been evaluated.

2. Materials and Methods

2.1 Animals and clinical records

The Ethical Committee of the University of Turin (Commissione di Etica e Benessere Animale del Dipartimento di Scienze Veterinarie di Torino) approved the study with protocol number 311/21. Twenty Ragusana crossbreed jennies and their 20 newborn foals, housed on a farm intended for the production of organic milk for human consumption and cosmetics, were enrolled in the study.

The jennies were divided into different categories on the basis of:

- *season*, classifying the jennies into three groups according to the date of delivery (spring, summer and autumn);
- *age*, dividing the animals into 'younger' (up to 10 years) and 'older' (over 10 years);
- *parity*, separating the jennies into primiparous and pluriparous.

It should be clarified that 'younger' and 'primiparous' jennies may overlap in part because this research has been conducted on animals kept in a farm which breeds donkeys for commercial purposes (milk production for human consumption). Therefore, in order to maintain productivity levels compatible with the commercial activity, there are no animals over 10 years of age that are primiparous since the jennies, resulting from internal replacement, start breeding at 3-4 years.

The mean age was 9.5±4.9 years (±Standard Deviation; median: 8 years; range: 3-19 years; mode: 8 years). Sixteen jennies (80%) were pluriparous and four (20%) were primiparous

(mode: pluriparous). Thirteen (65%) were under 10 years of age, 7 (35%) were 10 years old or older (Table 1) ***[Table 1 near here]***.

The jennies had been subjected to natural assisted mating on alternate days, based on the behavioural signs of oestrus identified visually and confirmed by ultrasound examination. The pregnancies were monitored by weekly ultrasound, from 14 to 35 days, and monthly up to the 12th month. Data on the pregnancy duration, time of delivery and jennies' health conditions in the days before and after parturition were collected. To determine the term of pregnancy, the size of the mammary gland, milk secretion, the appearance of the external genitalia, the position of the tail, the abdominal profile and, in general, the attitude of the animals were evaluated. The clinical data were supported by the farm calendar in which the expected date of delivery for each donkey, calculated on the basis of the stable average (370 days) from the date of the last mating, was reported. In the days immediately preceding and following parturition, the jennies were kept in a delivery room, where a 24/24h monitoring with a wireless camera was performed.

In the immediate post-partum period, a Basic Physical Examination (BPE) of the foals, the disinfection of the umbilical cord and verification of colostrum intake were performed. Foals' BPE was repeated daily for the first week post-partum.

2.2. Blood and mammary secretion sampling

One colostrum and one blood sample from each jenny, and one blood sample from each newborn, for a total of 60 samples, were collected (20 colostrum samples; 20 jennies' blood serum samples; 20 foals' blood serum samples).

Milking was performed manually within 1 hour after foaling and always before the foal got up for the first feed. At least 5 mL of colostrum were milked from both nipples, previously

cleaned, and collected in a 50 mL Falcon tube, after eliminating the first drops. The sample was then split into multiple 1.5 mL Eppendorf tubes.

Twenty-four hours post-partum, a blood sample was collected (9 mL) from each jenny and foal from the jugular vein, using a Vacutainer® tube. The serum was obtained by centrifugation (1,000 g for 10') and divided into several 1.5 mL Eppendorf tubes.

All samples were identified and immediately refrigerated, then frozen at -20° C until analysis, carried out in the laboratory of the University Veterinary Hospital (OVU) of the University of Turin (Italy).

2.3 Total Proteins and γ-globulins Analysis

The total proteins (TP) contained in each sample were determined using a hand-held Reichert Vet 360 optical refractometer (Reichert Technologies, Buffalo, New York, USA), in accordance with Elsohaby et al. (2018), who demonstrated that in horse foals the serum TP concentration measured with this technique is positively correlated with the RID-Ig.

Hydrasys (Sebia Italia S.r.l., Bagno a Ripoli, Florence, Italy), a semi-automatic multiparametric instrument, with Hydragel Protein(E) 15/30 agarose gel was used to quantify colostral and serum Ig. The instrument automatically performed the electrophoretic migration, washing, drying and colouring of the gel that was placed in the scanner for the densitometric reading of the protidogram thereafter. The relative concentration of each protein fraction was interpreted as a percentage of the optical absorption, based on the absolute concentration (g/L) of the TP of the sample. The electrophoretic curves were read and possibly corrected using the Phoresis software (Sebia Electrophoresis®, Sebia Italia S.r.l., Bagno a Ripoli, Florence, Italy). The percentage and g/L values of Electrophoretic Gamma Globulins (EGG) were obtained.

2.4 Statistical Analysis

All data underwent descriptive statistics.

The normality of the data distribution was assessed with the Kolmogorov-Smirnov test.

The differences in pregnancy length and foals' weight in relation to the sex of the foetus were analysed by means of independent sample t-test, while a possible relationship between pregnancy length and foals' weight was investigated with Pearson correlation.

The mean, standard deviation, median and range of total protein (TP) and γ-globulin concentration in jennies' and newborns' serum and colostrum were calculated.

The presence of a possible correlation between TP and γ-globulin concentrations and between the TP as well as the γ-globulin content in the different matrices (foals' and jennies' sera and colostrum) were investigated with Pearson's or Spearman's tests according to the data distribution.

The different factors (*season*, *age* and *parity*) that could have influenced the serum and colostral TP and γ-globulin concentrations were analysed.

The analysis of variance or the Kruskal Wallis test were performed to investigate the differences between the TP and γ-globulin levels over the seasons.

Similarly, to compare the concentrations of TP and γ-globulin in relation to age and parity, independent sample t-test or the Mann–Whitney U test were applied.

A possible association between each factor described above and the quality of the colostrum as well as the efficiency of the transfer of passive immunity to the foal were investigated. For this analysis, the samples were divided into groups according to the cut-off values established for the mare (Cash 1999; Tscheschlok et al. 2017). More in details, the colostrum samples have been divided into 4 groups, based on the γ-globulin concentration (Cash 1999): 'very good' quality (IgG > 80 g/L), 'good' quality (IgG between 50 and 80 g/L), 'fair' quality (IgG between 28 and 50 g/L) and 'poor' quality colostra (IgG < 28 g/L). The transfer of passive

175 immunity has been indicated as ‘complete’ when the concentration of γ-globulin in foal
176 serum was > 8 g/L, while a partial failure of passive immunity transfer (PFPT) has been
177 considered when γ-globulin in foal serum was between 4 and 8 g/L and failure of passive
178 transfer (FPT) with γ-globulin concentration < 4 g/L (Tscheschlok et al. 2017). To evaluate
179 the pairs of considered variables, Fisher's exact test was used.
180 All the analyses were performed with IBM SPSS Statistics for Mac, Version 27 (Armonk,
181 NY: IBM Corp.). Differences were considered statistically significant when $p<0.05$, whereas
182 for p values between 0.05 and 0.1 a tendency towards significance was considered.
183
184 **3. Results**
185 ***3.1 Clinical findings***
186 All the jennies had a normal pregnancy and peripartum period, and none of the foals showed
187 any signs of neonatal disease within the first week of life.
188 The mean pregnancy length was 375.15±13.18 days (median: 373 days; range: 350-398 days)
189 (Table 1), with slight differences related to the sex of the foetus (male foetuses: mean
190 377.1±11.41 days, median: 375 days, range: 362-395 days; female foetuses: mean
191 373.2±15.11 days, median: 369.50 days, range: 350-398 days), but without statistical
192 significance.
193 The foals were 10 males and 10 females, with an average birth weight of 30.43±5.02 kg
194 (median: 28.9 kg; range: 24.5-41.1 kg) (Table 1). Mean and standard deviation of birth
195 weights for females and males were 31.26±6.10 kg (median: 28.80 kg; range: 24.50-41.10
196 kg) and 29.60±3.83 kg (median: 29.25 kg; range: 24.50-37.00 kg), respectively, but the
197 difference was not statistically significant. No significant correlation has been found between
198 the pregnancy length and the foals’ weight (r=0.14).

199 Eight deliveries occurred in spring (40%), 8 in summer (40%) and 4 in autumn (20%). No
200 births took place in winter (Table 1).
201 Twelve jennies (60%) gave birth between 1 and 4 am; 5 (25%) between 3 and 5 pm; 2 (10%)
202 between 9 and 11 pm; 1 (5%) at 7 am (Table 1).
203 Eighteen foals (90%) stood up and spontaneously took the colostrum within around 1 hour of
204 foaling. One (5%) took about 3 hours to start feeding and another one (5%) needed assistance
205 because the jenny (primiparous) initially refused it.
206
207 ***3.2 Total Proteins***
208 The TP values in jennies' and foals' sera and in the colostrum are reported in Table 2. ***[Table***
209 ***2 near here]***.
210 The content of TP in the different samples (serum of jennies, foals and colostrum) did not
211 vary significantly in relation to age or parity, whereas statistically significant seasonal
212 differences were found in TP concentration of jennies' serum (spring: 69.63±4.93 g/L,
213 summer: 71.63±2.97 g/L, autumn: 79.50±6.66 g/L; $p<0.05$).
214 There was no significant correlation between jennies' serum and colostrum TP concentration
215 ($r=0.41$), nor between the concentration in jennies' and foals' sera ($\rho=0.42$; Table 3).
216 Moreover, no statistically significant correlation was found between foals' serum TP
217 concentration and colostral one ($\rho=0.30$), whereas positive and statistically significant
218 correlations were observed between TP and γ-globulin content in colostrum ($r=0.69$, $p<0.01$)
219 and both in the jennies' ($\rho=0.50$, $p<0.05$) and foals' sera ($\rho=0.70$, $p<0.01$; Table 3) ***[Table 3***
220 ***near here]***.
221
222 ***3.3 γ-globulin***
223 *3.3.1 Concentration of γ-globulin in jennies' sera*

γ-globulin concentrations in jennies' sera are reported in Table 4 ***[Table 4 near here]***.

The levels of γ-globulin in the jennies' sera did not change significantly in the different seasons examined (spring: 17.34±2.27 g/L, summer: 18.07±2.04 g/L, autumn: 21.95±6.53 g/L), nor on the basis of age (younger: 18.65±3.62 g/L, older: 18.39±3.99 g/L), or parity (primiparous: 17.85±1.01 g/L, pluriparous: 18.73±4.06 g/L).

3.3.2 Concentration of γ-globulin in foals' sera

γ-globulin concentrations in foals' sera are reported in Table 4.

Season of birth (spring: 11.07±5.94 g/L, summer: 10.16±2.69 g/L, autumn: 12.22±8.21 g/L), jennies' age (younger: 10.71±5.05 g/L, older: 11.37±5.83 g/L) and parity (primiparous: 8.90±3.48 g/L, pluriparous: 11.45±5.51 g/L) did not significantly affect serum γ-globulin concentration in newborns.

Based on the classification established for the horse (Tscheschlok et al. 2017), 14 of 20 donkey foals (70%) had received a complete transfer of the passive immunity, with concentrations of γ-globulin > 8 g/L (mean: 13.15±4.60 g/L; median: 11.15 g/L; mode: 5.00-12.00 g/L; range: 8.30-23.70 g/L). The other 6 foals (30%) had a PFPT (mean γ-globulin concentration: 5.78±1.29 g/L; median: 5.75 g/L; mode: 4.00-6.00 g/L; range: 4.20-7.90 g/L). FPT (γ-globulin < 4 g/L) was not found in any newborn.

Season of birth, age and parity of the jennies were not statistically associated with the effectiveness of the transfer, either complete or partial, even if a lower incidence of PFTP has been observed in summer, although in the absence of statistical evidence.

3.3.3 γ-globulin in colostrum

γ-globulin concentrations in the colostra are reported in Table 4.

Concentration of γ-globulin in the colostrum did not vary significantly in relation to the age (younger: 79.85±29.71 g/L, older: 57.22±16.13 g/L) and parity (primiparous: 76.22±22.78 g/L, pluriparous: 70.86±29.25 g/L) of the jennies nor in relation to the season (spring: 58.78±17.12 g/L, summer: 83.21±19.87 g/L, autumn: 75.67±49.02 g/L).

Classification of colostrum samples according to γ-globulin content, on the basis of the qualitative categories established for the mare (Cash 1999), showed a majority of high-quality samples: 6 colostra (30%) were of 'very good' quality (IgG > 80 g/L; average: 105.33±22.78 g/L, median: 100.22 g/L, mode 80-110 g/L, range: 84.59-146.75 g/L), 11 colostra (55%) were of 'good' quality (IgG between 50 and 80 g/L; mean: 62.74±9.73 g/L, median: 56.50 g/L, mode: 50.00-60.00 g/L, range: 52.40-79.90 g/L), 3 colostra (15%) were of 'fair' quality (IgG between 28 and 50 g/L; mean: 38.82 ± 3.91 g/L, median: 38.20 g, mode: 34.00-39.00 g/L, range: 35.26-43.00 g/L). No 'poor' quality colostra have been found (IgG < 28 g/L).

Colostrum quality (very good, good or fair) was significantly associated with the efficiency of the transfer of passive immunity (complete or PFPT) ($p<0.05$). However, considering the quality of the colostra in the examined seasons, the good quality colostra were distributed in a similar way in spring and summer, but among the 6 colostra containing more than 80 g/L of γ-globulin, 4 were collected in summer (66.6%), 1 in spring (16.7%) and 1 in autumn (16.7%).

3.3.4 Correlation between γ-globulin in the different samples

There was no significant correlation between jennies' serum and colostrum γ-globulin concentration ($\rho=0.29$), nor between the jennies' and newborns' sera ($\rho=0.05$), but a positive and statistically significant correlation has been found between the γ-globulin concentration in the foal's serum and colostrum ($r=0.53$, $p<0.05$; Table 3).

4. Discussion

Little is known about the transfer of passive immunity in the donkey foal (Veronesi et al. 2014; Turini et al. 2020a; Turini et al. 2020b). To the best of our knowledge, there are no works that have evaluated the γ-globulin content of colostrum and maternal and neonatal serum in relation to birth season, age and parity of the jennies. Factors such as age and reproductive season have only been studied for their influence on the duration of pregnancy, on various aspects of the oestrous cycle in this species (Galisteo and Perez-Marin 2010) and on the quality of the milk intended for human consumption (D'Alessandro et al. 2011; Cosentino et al. 2012; Bordonaro et al. 2013; Martini et al. 2014; Martini et al. 2018).

The animals included in this study showed uneventful pregnancies and natural vaginal deliveries. The very wide age range of the jennies reflects the age distribution on the farm. The mean pregnancy duration was in line with the stud farm average and comparable to that reported by other authors (Fielding 1988; Meira et al. 1998; Tosi et al. 2013; Carluccio et al. 2015). Also, the trend towards a longer gestation in case of a male foetus is in agreement with previous studies (Carluccio et al. 2015). The mean birth weight of the donkey foals is similar to that reported by other authors (Carluccio et al. 2008; Veronesi et al. 2010; Veronesi et al. 2014; Turini et al. 2020a).

Most of the jennies gave birth at night, in accordance with what is described for the mare (Christensen 2011). In addition, the first feed within 1 hour of foaling is comparable to the mare's foal (Sellon 2006).

Applying the cut-offs defined by Cash (Cash 1999), none of the foals in this study appeared to be affected by PFPT or FPT. Coherently with the reliability of these cut-offs for the donkey species, none of the foals with PFPT showed clinical signs of neonatal pathologies.

297 Having shown a positive and statistically significant correlation between TP and γ-globulin
298 content in the colostrum, the optical refractometer could also be used in the field to select the
299 best quality colostra to be collected for the creation of a farm colostral bank. Even in this
300 case, however, it would be necessary to validate cut-offs to define the quality of the
301 colostrum in the donkey, currently estimated mainly using the Brix refractometer (Turini et
302 al. 2020b).
303 Although the radioimmunodiffusion assay (RID) is the gold standard for the diagnosis of
304 failure of passive immunity transfer in the equine species, increasing numbers of researchers
305 have been considering the possibility of replacing it with electrophoresis (Rumbaugh et al.
306 1978). Electrophoresis does not depend on standard curves and may be more accurate than
307 the single radial immunodiffusion assay, that shows variability in the results depending on
308 the commercial test used (Metzger et al. 2006). However, so far, few studies have
309 investigated its usefulness in the field in mares (Tscheschlok et al. 2017).
310 While RID measures IgG concentration, electrophoresis measures the non-specific fraction of
311 the γ-globulins. The two values do not show a perfect, but adequate agreement. The
312 difference between IgG-RID and EGG (Electrophoretic Gamma Globulins) is more evident
313 for high values (at serum concentrations >8 g/L), when the diagnosis of FPT is not
314 compromised (Tscheschlok et al. 2017). Probably, this difference between the two methods is
315 due to the fact that Ig can migrate not only in the γ-globulin fraction, but also in the β2-
316 globulin fraction, and for this reason the IgG-RID may provide a higher concentration
317 (Makimura et al. 1975; Rumbaugh et al. 1978).
318 In our work, and in accordance with literature, no relationships have been identified between
319 the γ-globulin content in the serum of the jennies and the examined parameters (season, age
320 and parity).

To date, few papers have been published on IgG serum concentration of the donkey foal (Veronesi et al. 2014; Turini et al. 2020a). Our values are slightly higher (10.94±5.19 g/L) than those observed by Veronesi et al. (2014) (8 g/L, 12 h after birth), but in line with those reported by Turini et al. (2020a) (14.91±0.50 g/L, 24 h after birth). The difference among the studies could be due to several reasons: our sample size was larger and the diagnostic method was different. In this research jennies were crossbred, while the other studies referred to purebred animals, Martina Franca (Veronesi et al. 2014) and Amiata (Turini et al. 2020a). Finally, as reported by Turini et al. (2020a), the fact that in Veronesi's et al. work (Veronesi et al. 2014) the jennies had been milked in the days before giving birth, could have slightly influenced the post-foaling colostral quality.

In our work, 30% of the foals had PFPT, based on the classification established for the horse (Tscheschlok et al. 2017), however all were apparently healthy in the days following parturition, showing normal neonatal development. We did not observe FPT and even foals with a very low IgG content (IgG < 1.8 g/L) at 24-48 hours showed no signs of pathology, as reported by Veronesi et al. (2014). The association between a very low γ-globulin concentration and the absence of clinically evident neonatal diseases is extremely anomalous. The hypothesis that can be formulated is that the minimum antibody coverage that the donkey foal requires in the first days of life is lower than that needed by the horse foal, also because, in donkeys, the non-specific immunity provided by lysozyme, very abundant in colostrum and in donkey milk, seems to play a key role (Qureshi and Enbergs 2012; Veronesi et al. 2014). It would be interesting to evaluate serum IgG concentration of pathological foals to understand if the cut-off for defining the failure of passive immunity transfer in the donkey is different from that established for the horse.

344 Moreover, using TPs instead of γ-globulin as an indicator of FPT, according to the cut-offs
345 defined by Elsohaby et al. (Elsohaby et al. 2018), none of the foals in this research would
346 have presented PFPT.
347 The transfer of passive immunity (complete or partial) was not affected by birth season, age
348 and parity of the jenny or by the quality of colostrum. In the mare, the incidence of FPT
349 appears to be lower in spring, in accordance with the physiological reproductive season of the
350 species (Clabough et al. 1991). Foals born between December and March in the Northern
351 hemisphere are more predisposed to develop FPT than those born in months with longer
352 daylight hours (Le Blanc et al. 1992). This trend is in agreement with what has long been
353 observed in the bovine species (Donovan et al. 1986) and may be equally valid for the
354 donkey, which has an increasing photoperiod polyestral cyclicity like the mare, but with a
355 lower seasonality, especially in temperate climates (Wilborn and Pugh 2011).
356 As known for mares, our study showed that also for the donkey species the incidence of FPT
357 is not higher in foals born to aged jennies. However, with advancing age, the fertility
358 decreases and physiological changes may affect foal development or nursing abilities
359 (Clabough et al. 1991). Nevertheless, a limitation of our study is that the jennies were
360 divided into older and younger ones, using 10 years as the cut-off value, but only one was
361 actually elderly (19 years).
362 Parity did not affect the transfer of passive immunity, although the primiparous accounted for
363 only 20% of the animals. In mares as well, no differences have been reported in the incidence
364 of FPT between foals born to maidens or to pluriparous animals (Clabough et al. 1991;
365 Raidal 1996).
366 All the jennies in the study produced colostrum with a γ-globulin concentration higher than
367 the 29.5 g/L reported by Veronesi et al. (2014) and similar to those found by Turini et al.
368 (2020a), albeit with considerable individual differences (mean: 71.93±27.61 g/L).

369 The age of the jennies did not seem to influence colostral γ-globulin content, although the
370 colostrum of the younger jennies tended ($0.05<p<0.1$) to be richer in comparison with that of
371 the older animals, in agreement to what has been described in the mare (Clabough et al.
372 1991). Parity did not show any significant effect on the colostral γ-globulin content which,
373 however, was slightly higher in primiparous than in pluriparous. This observation is in
374 contrast to what has been observed in the mare, where primiparous are more likely to show
375 lower quality colostrum (Clabough et al. 1991). Before putting forward the hypothesis of a
376 difference between the two species, it should be considered that only 4 jennies in this study
377 were primiparous, which is a number too small to generate representative results.
378 According to the classification in use in the mare (Cash 1999), 55% of the donkey colostra in
379 this research were of good quality, 30% were excellent, 15% fair. None was of poor quality.
380 No associations have been found between the colostrum quality and the season, age and
381 parity of the jennies. It is also possible that the classification of donkey colostrum into
382 qualitative categories should be different from that of the mare, species for a much wider
383 literature is available. To answer these questions, it would be important to examine a greater
384 number of animals. However, observing the quality of the colostra in the three seasons
385 examined, the distribution followed an interesting trend: the good quality colostra were
386 distributed in a similar way in the spring and summer months, but among the 6 colostra
387 containing more than 80 g/L of γ-globulin, 4 were produced in summer (66.6%), 1 in spring
388 (16.7%) and 1 in autumn (16.7%). Also in summer, the incidence of PFPT in foals was lower,
389 although in the absence of statistical evidence. This result highlights a possible disagreement,
390 compared to the mare in which the effectiveness of the transfer of immunity to the foal and
391 the quality of the colostrum are better in spring (Clabough et al. 1991).
392 This could be traced back to the evolutionary origins of the two species: the horse originates
393 from the Eurasian prairies and can withstand low temperatures without problems. Instead,

394 the domestic donkey is native to the African deserts and, despite its remarkable ability to
395 adapt, it is an animal that prefers a warm and dry climate (Senior 2013).
396 Indeed, a breeding management that avoid births to take place in winter is applied, in order to
397 prevent the exposure of the newborns to extremely low temperatures.
398 While jennies' γ-globulin concentrations in serum and colostrum were not significantly
399 correlated, and neither were the jennies' and foals' γ-globulin serum concentrations,
400 analogously to what described in the horse (Morris et al. 1985; Kohn et al. 1989), a
401 statistically significant positive correlation was found between the foals' γ-globulin levels in
402 serum and colostrum. The foal serum concentration reflects the concentration of γ-globulin
403 that it receives from the colostrum. In the horse, these two parameters have been associated
404 with discordant results (Morris et al. 1985; Kohn et al. 1989; Erhard et al. 2001) showing
405 from poor but significant correlations (Morris et al. 1985; Kohn et al. 1989) to no correlation
406 (Erhard et al. 2001).
407 The positive correlation between the values implies that, considering a colostrum of adequate
408 quality and a healthy foal, breastfeeding is more than suitable for a correct transfer of
409 immunity; however, in case of a colostrum with low γ-globulins level, supplementation is
410 necessary. In horse studs, colostral IgG concentrations are usually evaluated immediately
411 after foaling (Slovis and Vaala 2011), by measuring TPs with a refractometer (McCue 2014)
412 and this practice could be usefully adopted also for donkeys.
413 Moreover, for this reason, it would be essential to establish cut-offs regarding the quantity of
414 colostral and neonatal γ-globulins suitable for the species, since, according to this study and
415 that of Veronesi et al. (Veronesi et al. 2014), it seems that γ-globulin concentrations in the
416 foals' sera which are considered low for horses are not so low for donkeys. In any case, it is
417 also advisable for a donkey farmer to create a colostrum bank with the best quality colostra to
418 thaw and administer orally if needed (Vivrette 2011; Turini et al. 2020b).

5. Conclusions

The transfer of passive immunity from jenny to foal is still largely unknown and, currently, there are no in-depth studies on the factors that can influence this delicate immune function. Apparently, this process is similar in horses and donkeys. However, it would be interesting to investigate the relationship between the donkey's seasonality and its reproductive activity, referring to a larger population of animals. Comparing the donkey to the horse there is, in fact, the risk of not grasping the subtle differences that make the breeding of these two species completely different.

Declarations of interest statements

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author, [A.B.], upon reasonable request.

References

- Bernard WV, Barr BS. 2012. Immunologic and hematologic disorders. Equine pediatric medicine. 1st ed. London: Barr-Manson Publishing; p. 72-90.
- Bordonaro S, Dimauro C, Criscione A, Marletta D, Macciotta NP. 2013. The mathematical modeling of the lactation curve for dairy traits of the donkey (Equus asinus). J Dairy Sci. 96(6):4005-4014.
- Bough J. 2011. Equus asinus: Origins, Domestication, Breeds and Characteristics. Donkey. Chicago: University of Chicago Press.

- Brinsko S, Blanchard T, Varner D, Schumacher J, Love C, Hinrichs K, Hartman D. 2010. Routine Management of the Neonatal Foal. Manual of equine reproduction. 3rd ed. Elsevier Science Health; p. 143-159.
- Carluccio A, Gloria A, Veronesi MC, De Amicis I, Noto F, Contri A. 2015. Factors affecting pregnancy length and phases of parturition in Martina Franca jennies. Theriogenology. 84(4):650-655.
- Carluccio A, Panzani S, Tosi U, Riccaboni P, Contri A, Veronesi MC. 2008. Morphological features of the placenta at term in the Martina Franca donkey. Theriogenology. 69(8):918-924.
- Cash RSG. 1999. Colostral quality determined by refractometry. Equine Vet Educ. 11(1):36-38.
- Christensen BW. 2011. Parturition. In: McKinnon AO SE, Vaala WE, Varner DD, editor. Equine reproduction. 2nd ed. Hoboken, New Jersey: Wiley-Blackwell; p. 2268-2276.
- Clabough DL, Levine JF, Grant GL, Conboy HS. 1991. Factors Associated with Failure of Passive Transfer of Colostral Antibodies in Standardbred Foals. J Vet Intern Med. 5(6):335-340.
- Cosentino C, Paolino R, Freschi P, Calluso AM. 2012. Short communication: jenny milk production and qualitative characteristics. J Dairy Sci. 95(6):2910-2915.
- D'Alessandro AG, Martemucci G, Jirillo E, De Leo V. 2011. Major whey proteins in donkey's milk: effect of season and lactation stage. Implications for potential dietary interventions in human diseases. Immunopharmacol Immunotoxicol. 33(2):259-265.
- Dai F, Dalla Costa E, Burden F, Judge A, Minero M. 2019. The development of guidelines to improve dairy donkey management and welfare. Ital J Anim Sci. 18(1):189-193.

- Donovan GA, Badinga L, Collier RJ, Wilcox CJ, Braun RK. 1986. Factors influencing passive transfer in dairy calves. J Dairy Sci. 69(3):754-759.
- Elsohaby I, Riley CB, McClure JT. 2018. Usefulness of digital and optical refractometers for the diagnosis of failure of transfer of passive immunity in neonatal foals. Equine Vet J. 51(4):451-457.
- Erhard MH, Luft C, Remler HP, Stangassinger M. 2001. Assessment of colostral transfer and systemic availability of immunoglobulin G in new-born foals using a newly developed enzyme-linked immunosorbent assay (ELISA) system. J Anim Physiol Anim Nutr (Berl). 85(5-6):164-173.
- Fielding D. 1988. Reproductive characteristics of the jenny donkey--Equus asinus: a review. Trop Anim Health Prod. 20(3):161-166.
- Galisteo J, Perez-Marin CC. 2010. Factors affecting gestation length and estrus cycle characteristics in Spanish donkey breeds reared in southern Spain. Theriogenology. 74(3):443-450.
- Jeffcott LB. 1972. Passive immunity and its transfer with special reference to the horse. Biol Rev. 47(4):439-464.
- Kohn CW, Knight D, Hueston W, Jacobs R, Reed SM. 1989. Colostral and serum IgG, IgA, and IgM concentrations in Standardbred mares and their foals at parturition. J Am Vet Med Assoc. 195(1):64-68.
- Kummer L, Govaere J, Egri B. 2018. Comparison of the reliability of snap foal Ig test, Gamma-Check E test, refractometry and electrophoresis for determining the immune status of newborn foals in the first hours of life. Acta Vet Hung. 66:573-586.
- Le Blanc MM, Tran T, Baldwin JL, Pritchard EL. 1992. Factors that influence passive transfer of immunoglobulins in foals. J Am Vet Med Assoc. 200(2):179-183.

493 • Lester GD. 2011. Colostrum: Assessment of quality and artificial supplementation. In:
494 McKinnon AO SE, Vaala WE, Varner DD, editor. Equine reproduction. 2nd ed.
495 Hoboken, New Jersey: Wiley-Blackwell; p. 342-345.
496 • Makimura S, Tomoda I, Usui K. 1975. Quantitative studies on immunoglobulins and
497 transferrin in equine serum. Nihon Juigaku Zasshi. 37(2):187-198.
498 • Martini M, Altomonte I, Licitra R, Salari F. 2018. Short communication:
499 Technological and seasonal variations of vitamin D and other nutritional components
500 in donkey milk. J Dairy Sci. 101(10):8721-8725.
501 • Martini M, Altomonte I, Salari F, Caroli AM. 2014. Short communication:
502 Monitoring nutritional quality of Amiata donkey milk: Effects of lactation and
503 productive season. J Dairy Sci. 97(11):6819-6822.
504 • McCue PM. 2014. Evaluation of Colostrum Quality: Brix Refractometry. In:
505 Dascanio J MP, editor. Equine Reproductive Procedures. Wiley-Blackwell; p. 297-
506 298.
507 • McKenzie HC. 2018. Disorders of Foals. In: Reed SM BW, Sellon DC, editor. Equine
508 Internal Medicine. 4th ed. Elsevier; p. 1365-1459.
509 • Meira C, Ferreira JCP, Papa FO, Henry M. 1998. Ultrasonographic evaluation of the
510 conceptus from days 10 to 60 of pregnancy in jennies. Theriogenology. 49(8):1475-
511 1482.
512 • Metzger N, Hinchcliff KW, Hardy J, Schwarzwald CC, Wittum T. 2006. Usefulness
513 of a Commercial Equine IgG Test and Serum Protein Concentration as Indicators of
514 Failure of Transfer of Passive Immunity in Hospitalized Foals. J Vet Intern Med.
515 20(2):382-387.
516 • Morris DD, Meirs DA, Merryman GS. 1985. Passive transfer failure in horses:
517 incidence and causative factors on a breeding farm. Am J Vet Res. 46(11):2294-2299.

- Perkins GA, Wagner B. 2015. The development of equine immunity: Current knowledge on immunology in the young horse. Equine Vet J. 47(3):267-274.
- Qureshi AS, Enbergs H. 2012. Studies on the lysozyme activity in the milk of female donkeys (Equus asinus) in relation to reproductive physiology. J Anim Plant Sci. 22:70-74.
- Raidal SL. 1996. The incidence and consequences of failure of passive transfer of immunity on a Thoroughbred breeding farm. Aust Vet J. 73(6):201-206.
- Rumbaugh GE, Ardans AA, Ginno D, Trommershausen-Smith A. 1978. Measurement of neonatal equine immunoglobulins for assessment of colostral immunoglobulin transfer: comparison of single radial immunodiffusion with the zinc sulfate turbidity test, serum electrophoresis, refractometry for total serum protein, and the sodium sulfite precipitation test. J Am Vet Med Assoc. 172(3):321-325.
- Sellon D. 2006. Neonatal Immunology. In: MR P, editor. Equine Neonatal Medicine: A Case-Based Approach. 1st ed. Elsevier Saunders; p. 29-38.
- Senior JM. 2013. Not small horses: improving treatments for donkeys. Vet Rec. 173(12):292-293.
- Slovis N, Vaala W. 2011. Diagnostic laboratory resources and critical care supplies. In: McKinnon AO SE, Vaala WE, Varner DD, editor. Equine reproduction. 2nd ed. Hoboken, New Jersey: Wiley-Blackwell; p. 830-837.
- Tosi U, Bernabò N, Verni F, Valbonetti L, Muttini A, Mattioli M, Barboni B. 2013. Postpartum reproductive activities and gestation length in Martina Franca jennies, an endangered Italian donkey breed. Theriogenology. 80(2):120-124.
- Tscheschlok L, Venner M, Howard J. 2017. Comparison of IgG concentrations by radial immunodiffusion, electrophoretic gamma globulin concentrations and total globulins in neonatal foals. Equine Vet J. 49(2):149-154.

- Turini L, Bonelli F, Nocera I, Battaglia F, Meucci V, Panzani D, Mele M, Sgorbini M. 2020a. Evaluation of jennies' colostrum: IgG concentrations and absorption in the donkey foals. A preliminary study. Heliyon. 6(8):e04598.
- Turini L, Nocera I, Bonelli F, Mele M, Sgorbini M. 2020b. Evaluation of Brix Refractometry for the Estimation of Colostrum Quality in Jennies. J Equine Vet Sci. 92:103172.
- Veronesi MC, Dall'Ara P, Gloria A, Servida F, Sala E, Robbe D. 2014. IgG, IgA, and lysozyme in Martina Franca donkey jennies and their foals. Theriogenology. 81(6):825-831.
- Veronesi MC, Villani M, Wilsher S, Contri A, Carluccio A. 2010. A comparative stereological study of the term placenta in the donkey, pony and Thoroughbred. Theriogenology. 74(4):627-631.
- Vivrette S. 2011. Assessment and modification of passive transfer. In: McKinnon AO SE, Vaala WE, Varner DD, editor. Equine reproduction. 2nd ed. Hoboken, New Jersey: Wiley-Blackwell; p. 346-352.
- Wilborn R, Pugh D. 2011. Donkey reproduction. In: McKinnon AO SE, Vaala WE, Varner DD, editor. Equine reproduction. 2nd ed. Hoboken, New Jersey: Wiley-Blackwell; p. 2835-2838.