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Analysis of factors influencing the transfer of passive immunity in the donkey foal

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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 ($r = 0.69$; $p < .01$), confirming the possibility to use this diagnostic tool in the field as a first, inexpensive approach for colostrum evaluation.

HIGHLIGHTS

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

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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 h after birth (McKenzie 2018). This drastic fall is due to the replacement of specialised 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

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destined to rapidly decrease (Brinsko et al. 2010). As a consequence, the foal should receive colostrum within the first 2 h after birth (Jeffcott 1972; Vivrette 2011). Within 4–6 h after ingestion, Ig enter the foal's bloodstream, peak 24–48 h 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 utilisation (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 h after birth, while regarding the donkey, the knowledge is currently very limited (Veronesi et al. 2014; Turini, Bonelli, et al. 2020a; Turini, Nocera, et al. 2020b) 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.

Materials and methods

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).

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 24/24 h monitoring with a wireless camera was performed.

Table 1. Clinical data of jennies and foals enrolled in the study.

ID	Age (years)	Age (category)	Parity	Season	Pregnancy length (days)	Time of parturition	Foal weight (kg)	Foal sex	Colostrum quality	Immunity transfer
1	15	Older	pluriparous	spring	383	evening	34.3	F	good	complete
2	9	Younger	pluriparous	spring	380	night	28.5	M	good	complete
3	3	Younger	primiparous	spring	395	morning	27.6	M	good	complete
4	6	Younger	pluriparous	spring	377	afternoon	33.6	M	fair	PFPT
5	3	Younger	primiparous	spring	373	night	30.8	M	good	PFPT
6	3	Younger	primiparous	summer	366	night	24.5	F	fair	PFPT
7	4	Younger	primiparous	summer	367	night	24.8	M	good	complete
8	8	Younger	pluriparous	summer	371	night	27.3	F	very good	complete
9	14	Older	pluriparous	summer	350	night	28.3	F	very good	complete
10	10	Younger	pluriparous	summer	368	night	41.1	F	good	PFPT
11	8	Younger	pluriparous	summer	383	night	37.0	M	good	complete
12	6	Younger	pluriparous	summer	395	night	30.0	M	good	complete
13	12	Older	pluriparous	autumn	366	afternoon	40.0	F	very good	complete
14	19	Older	pluriparous	autumn	373	night	31.2	M	very good	PFPT
15	14	Older	pluriparous	autumn	362	evening	28.0	M	very good	complete
16	17	Older	pluriparous	spring	366	afternoon	24.5	M	good	complete
17	8	Younger	pluriparous	spring	398	night	35.8	F	very good	complete
18	16	Older	pluriparous	spring	396	night	27.0	F	good	complete
19	7	Younger	pluriparous	summer	373	afternoon	25.0	F	good	complete
20	8	Younger	pluriparous	autumn	361	afternoon	29.3	F	fair	PFPT

Age (category): 'younger' $\leq$ 10 years, 'older' $>$ 10 years; Time of parturition: morning= 7 am, afternoon= 3–5 pm, evening= 9–11 pm, night= 1–4 am; Foal sex: F = female, M = male; Colostrum quality: 'very good'= IgG $>$ 80 g/L, 'good'= IgG between 50 and 80 g/L, 'fair'= IgG between 28 and 50 g/L; Immunity transfer: 'complete'= γ-globulin concentration in foal serum $>$ 8 g/L, 'PFPT' (Partial failure of passive immunity transfer)= γ-globulin concentration in foal serum between 4 and 8 g/L.

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.

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 h 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 (1000 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).

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. (2019), 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.

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 immunity has been indicated as 'complete' when the concentration of γ-globulin in foal serum was >8 g/L, while a partial failure of passive immunity transfer (PFPT) has been considered when γ-globulin in foal serum was between 4 and 8 g/L and failure of passive transfer (FPT) with γ-globulin concentration <4 g/L (Tscheschlok et al. 2017). To evaluate the pairs of considered variables, Fisher's exact test was used.

All the analyses were performed with IBM SPSS Statistics for Mac, Version 27 (Armonk, NY: IBM Corp.). Differences were considered statistically significant when $p < .05$, whereas for p values between .05 and .1 a tendency towards significance was considered.

Results

Clinical findings

All the jennies had a normal pregnancy and peripartum period, and none of the foals showed any signs of neonatal disease within the first week of life.

The mean pregnancy length was 375.15 ± 13.18 days (median: 373 days; range: 350–398 days) (Table 1), with slight differences related to the sex of the foetus (male foetuses: mean 377.1 ± 11.41 days, median: 375 days, range: 362–395 days; female foetuses: mean 373.2 ± 15.11 days, median: 369.50 days, range: 350–398 days), but without statistical significance.

The foals were 10 males and 10 females, with an average birth weight of 30.43 ± 5.02 kg (median: 28.9 kg; range: 24.5–41.1 kg) (Table 1). Mean and standard deviation of birth weights for females and males were 31.26 ± 6.10 kg (median: 28.80 kg; range: 24.50–41.10 kg) and 29.60 ± 3.83 kg (median: 29.25 kg; range: 24.50–37.00 kg), respectively, but the difference was not statistically significant. No significant correlation has been found between the pregnancy length and the foals' weight ($r = 0.14$).

Eight deliveries occurred in spring (40%), 8 in summer (40%) and 4 in autumn (20%). No births took place in winter (Table 1).

Twelve jennies (60%) gave birth between 1 and 4 am; 5 (25%) between 3 and 5 pm; 2 (10%) between 9 and 11 pm; 1 (5%) at 7 am (Table 1).

Eighteen foals (90%) stood up and spontaneously took the colostrum within around 1 h of foaling. One (5%) took about 3 h to start feeding and another one (5%) needed assistance because the jenny (primiparous) initially refused it.

Total proteins

The TP values in jennies' and foals' sera and in the colostrum are reported in Table 2.

The content of TP in the different samples (serum of jennies, foals and colostrum) did not vary significantly in relation to age or parity, whereas statistically significant seasonal differences were found in TP concentration of jennies' serum (spring: 69.63 ± 4.93 g/L, summer: 71.63 ± 2.97 g/L, autumn: 79.50 ± 6.66 g/L; $p < .05$).

There was no significant correlation between jennies' serum and colostrum TP concentration ($r = 0.41$),

nor between the concentration in jennies' and foals' sera ($\rho = 0.42$; Table 3). Moreover, no statistically significant correlation was found between foals' serum TP concentration and colostral one ($\rho = 0.30$), whereas positive and statistically significant correlations were observed between TP and γ-globulin content in colostrum ($r = 0.69$, $p < .01$) and both in the jennies' ($\rho = .50$, $p < .05$) and foals' sera ($\rho = 0.70$, $p < 0.01$; Table 3).

γ-Globulin

Concentration of γ-globulin in jennies' sera.

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

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

Table 2. Total protein (g/L) in the jennies' and foals' sera and in the colostra.

	Mean ± SD	Median	Range	*N*
Jennies' sera TP (g/L)				
Total	72.40 ± 5.77	71.50	62.00–85.00	20
Age				
Younger	71.15 ± 5.01[a]	71.00	62.00–80.00	13
Older	74.71 ± 6.75[a]	73.00	67.00–85.00	7
Parity				
Primiparous	70.50 ± 4.20[a]	70.50	66.00–75.00	4
Pluriparous	72.87 ± 6.12[a]	71.50	62.00–85.00	16
Season				
Spring	69.63 ± 4.93[a]	70.00	62.00–75.00	8
Summer	71.63 ± 2.97[a]	71.00	68.00–78.00	8
Autumn	79.50 ± 6.66[b]	81.50	70.00–85.00	4
Foals' sera TP (g/L)				
Total	55.25 ± 7.59	51.50	45.00–71.00	20
Age				
Younger	54.31 ± 7.26[a]	51.00	45.00–68.00	13
Older	57.00 ± 8.47[a]	55.00	47.00–71.00	7
Parity				
Primiparous	51.50 ± 4.12[a]	51.00	47.00–57.00	4
Pluriparous	56.19 ± 8.06[a]	53.50	45.00–71.00	16
Season				
Spring	53.50 ± 9.61[a]	49.00	45.00–71.00	8
Summer	54.25 ± 4.43[a]	51.50	51.00–62.00	8
Autumn	60.75 ± 7.27[a]	62.00	51.00–68.00	4
Colostral TP (g/L)				
Total	202.95 ± 77.40	183.50	114.00–412.00	20
Age				
Younger	205.92 ± 83.64[a]	179.00	114.00–412.00	13
Older	197.43 ± 70.21[a]	189.00	120.00–324.00	7
Parity				
Primiparous	174.50 ± 43.47[a]	183.50	114.00–217.00	4
Pluriparous	210.06 ± 83.32[a]	179.50	120.00–412.00	16
Season				
Spring	157.38 ± 42.94[a]	146.50	114.00–226.00	8
Summer	226.63 ± 89.42[a]	188.50	151.00–412.00	8
Autumn	246.75 ± 73.49[a]	255.50	152.00–324.00	4

Within groups ('Age', 'Parity' and 'Season'), [a]means without a common superscript letter differ at $p < .05$.

Table 4. γ-globulin (g/L) in the jennies' and foals' sera and in the colostra.

	Mean ± SD	Median	Range	*N*
Jennies' sera γ-globulin (g/L)				
Total	18.55 ± 3.65	17.55	14.10–29.30	20
Age				
Younger	18.65 ± 3.62[a]	17.80	14.50–29.30	13
Older	18.39 ± 3.99[a]	16.50	14.10–25.60	7
Parity				
Primiparous	17.85 ± 1.01[a]	17.75	16.80–19.10	4
Pluriparous	18.73 ± 4.06[a]	17.55	14.10–29.30	16
Season				
Spring	17.34 ± 2.27[a]	18.10	14.10–20.40	8
Summer	18.07 ± 2.04[a]	17.30	16.00–21.70	8
Autumn	21.95 ± 6.53[a]	21.05	16.40–29.30	4
Foals' sera γ-globulin (g/L)				
Total	10.94 ± 5.19	10.50	4.20–23.70	20
Age				
Younger	10.71 ± 5.05[a]	9.80	4.80–23.70	13
Older	11.37 ± 5.83[a]	10.60	4.20–21.30	7
Parity				
Primiparous	8.90 ± 3.48[a]	8.85	4.80–13.10	4
Pluriparous	11.45 ± 5.51[a]	10.65	4.20–23.70	16
Season				
Spring	11.07 ± 5.94[a]	9.25	4.80–21.30	8
Summer	10.16 ± 2.69[a]	10.90	5.60–13.40	8
Autumn	12.22 ± 8.21[a]	10.50	4.20–23.70	4
Colostral γ-globulin (g/L)				
Total	71.93 ± 27.61	63.75	35.26–146.75	20
Age				
Younger	79.85 ± 29.71[a]	79.90	43.00–146.75	13
Older	57.22 ± 16.13[a]	56.50	35.26–77.74	7
Parity				
Primiparous	76.22 ± 22.78[a]	84.85	43.00–92.18	4
Pluriparous	70.86 ± 29.25[a]	59.28	35.26–146.75	16
Season				
Spring	58.78 ± 17.12[a]	56.30	38.20–89.80	8
Summer	83.21 ± 19.87[a]	82.24	56.10–110.42	8
Autumn	75.67 ± 49.02[a]	60.33	35.26–146.75	4

Within groups ('Age', 'Parity' and 'Season'), means without a common superscript letter differ at $p < .05$.

Table 3. Correlation table.

	Colostral TP	Jennies' sera TP	Foals' sera TP	Colostral γ-globulin	Jennies' sera γ-globulin	Foals' sera γ-globulin
Colostral TP	1	$r = 0.41$ $p = .073$	$\rho = 0.30$ $p = .194$	$r = 0.69$ $p = .001$	–	–
Jennies' sera TP	$r = 0.41$ $p = .073$	1	$\rho = 0.42$ $p = .069$	–	$\rho = 0.5$ $p = .024$	–
Foals' sera TP	$\rho = 0.30$ $p = .194$	$\rho = 0.42$ $p = .069$	1	–	–	$\rho = 0.7$ $p = .001$
Colostral γ-globulin	$r = 0.69$ $p = .001$	–	–	1	$\rho = 0.29$ $p = .221$	$r = 0.53$ $p = .016$
Jennies' sera γ-globulin	–	$\rho = 0.5$ $p = .024$	–	$\rho = 0.29$ $p = .221$	1	$\rho = 0.05$ $p = .850$
Foals' sera γ-globulin	–	–	$\rho = 0.70$ $p = .001$	$r = 0.53$ $p = .016$	$\rho = 0.05$ $p = .850$	1

'*r*': correlation coefficient, 'ρ': Spearman's rho, '*p*' = significance.

parity (primiparous: 17.85 ± 1.01 g/L, pluriparous: 18.73 ± 4.06 g/L).

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.

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) ($p < .05$). 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%).

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 ($r = 0.53$, $p < .05$; Table 3).

Discussion

Little is known about the transfer of passive immunity in the donkey foal (Veronesi et al. 2014; Turini, Bonelli, et al. 2020a; Turini, Nocera, 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; 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, 2014; Turini, Bonelli, 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 h 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.

Having shown a positive and statistically significant correlation between TP and γ-globulin content in the colostrum, the optical refractometer could also be used in the field to select the best quality colostra to be collected for the creation of a farm colostral bank. Even in this case, however, it would be necessary to validate cut-offs to define the quality of the colostrum in the donkey, currently estimated mainly using the Brix refractometer (Turini, Nocera, et al. 2020b).

Although the radioimmunodiffusion assay (RID) is the gold standard for the diagnosis of failure of passive immunity transfer in the equine species, increasing numbers of researchers have been considering the possibility of replacing it with electrophoresis (Rumbaugh et al. 1978). Electrophoresis does not depend on standard curves and may be more accurate than the single radial immunodiffusion assay, that shows variability in the results depending on the commercial test used (Metzger et al. 2006). However, so far, few studies have investigated its usefulness in the field in mares (Tscheschlok et al. 2017).

While RID measures IgG concentration, electrophoresis measures the non-specific fraction of the γ-globulins. The two values do not show a perfect, but adequate agreement. The difference between IgG-RID and EGG (Electrophoretic Gamma Globulins) is more evident for high values (at serum concentrations >8 g/L), when the diagnosis of FPT is not compromised (Tscheschlok et al. 2017). Probably, this difference between the two methods is due to the fact that Ig can migrate not only in the γ-globulin fraction, but also in the β2-globulin fraction, and for this reason the IgG-RID may provide a higher concentration (Makimura et al. 1975; Rumbaugh et al. 1978).

In our work, and in accordance with literature, no relationships have been identified between the γ-globulin content in the serum of the jennies and the examined parameters (season, age and parity).

To date, few papers have been published on IgG serum concentration of the donkey foal (Veronesi et al. 2014; Turini, Bonelli, 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, Bonelli, 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, Bonelli, et al. 2020a). Finally, as reported by Turini, Bonelli, 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 h 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.

Moreover, using TPs instead of γ-globulin as an indicator of FPT, according to the cut-offs defined by Elsohaby et al. (Elsohaby et al. 2019), none of the foals in this research would have presented PFPT.

The transfer of passive immunity (complete or partial) was not affected by birth season, age and parity of the jenny or by the quality of colostrum. In the mare, the incidence of FPT appears to be lower in spring, in accordance with the physiological reproductive season of the species (Clabough et al. 1991). Foals born between December and March in the Northern hemisphere are more predisposed to develop FPT than those born in months with longer daylight hours (Le Blanc et al. 1992). This trend is in agreement with what has long been observed in the bovine species (Donovan et al. 1986) and may be equally valid for the donkey, which has an increasing photoperiod polyestral cyclicity like the mare, but with a lower seasonality, especially in temperate climates (Wilborn and Pugh 2011).

As known for mares, our study showed that also for the donkey species the incidence of FPT is not higher in foals born to aged jennies. However, with

advancing age, the fertility decreases and physiological changes may affect foal development or nursing abilities (Clabough et al. 1991). Nevertheless, a limitation of our study is that the jennies were divided into older and younger ones, using 10 years as the cut-off value, but only one was actually elderly (19 years).

Parity did not affect the transfer of passive immunity, although the primiparous accounted for only 20% of the animals. In mares as well, no differences have been reported in the incidence of FPT between foals born to maidens or to pluriparous animals (Clabough et al. 1991; Raidal 1996).

All the jennies in the study produced colostrum with a γ-globulin concentration higher than the 29.5 g/L reported by Veronesi et al. (2014) and similar to those found by Turini, Bonelli, et al. (2020a), albeit with considerable individual differences (mean: 71.93 ± 27.61 g/L).

The age of the jennies did not seem to influence colostral γ-globulin content, although the colostrum of the younger jennies tended ($.05 < p < .1$) to be richer in comparison with that of the older animals, in agreement to what has been described in the mare (Clabough et al. 1991). Parity did not show any significant effect on the colostral γ-globulin content which, however, was slightly higher in primiparous than in pluriparous. This observation is in contrast to what has been observed in the mare, where primiparous are more likely to show lower quality colostrum (Clabough et al. 1991). Before putting forward the hypothesis of a difference between the two species, it should be considered that only 4 jennies in this study were primiparous, which is a number too small to generate representative results.

According to the classification in use in the mare (Cash 1999), 55% of the donkey colostra in this research were of good quality, 30% were excellent, 15% fair. None was of poor quality.

No associations have been found between the colostrum quality and the season, age and parity of the jennies. It is also possible that the classification of donkey colostrum into qualitative categories should be different from that of the mare, species for a much wider literature is available. To answer these questions, it would be important to examine a greater number of animals. However, observing the quality of the colostra in the three seasons examined, the distribution followed an interesting trend: the good quality colostra were distributed in a similar way in the spring and summer months, but among the 6 colostra containing more than 80 g/L of γ-globulin, 4 were produced in summer (66.6%), 1 in spring (16.7%) and 1 in autumn (16.7%). Also in summer, the incidence of PFPT in foals was lower, although in the absence of statistical evidence. This result highlights a possible disagreement, compared to the mare in which the effectiveness of the transfer of immunity to the foal and the quality of the colostrum are better in spring (Clabough et al. 1991).

This could be traced back to the evolutionary origins of the two species: the horse originates from the Eurasian prairies and can withstand low temperatures without problems. Instead, the domestic donkey is native to the African deserts and, despite its remarkable ability to adapt, it is an animal that prefers a warm and dry climate (Senior 2013).

Indeed, a breeding management that avoid births to take place in winter is applied, in order to prevent the exposure of the newborns to extremely low temperatures.

While jennies' γ-globulin concentrations in serum and colostrum were not significantly correlated, and neither were the jennies' and foals' γ-globulin serum concentrations, analogously to what described in the horse (Morris et al. 1985; Kohn et al. 1989), a statistically significant positive correlation was found between the foals' γ-globulin levels in serum and colostrum. The foal serum concentration reflects the concentration of γ-globulin that it receives from the colostrum. In the horse, these two parameters have been associated with discordant results (Morris et al. 1985; Kohn et al. 1989; Erhard et al. 2001) showing from poor but significant correlations (Morris et al. 1985; Kohn et al. 1989) to no correlation (Erhard et al. 2001).

The positive correlation between the values implies that, considering a colostrum of adequate quality and a healthy foal, breastfeeding is more than suitable for a correct transfer of immunity; however, in case of a colostrum with low γ-globulins level, supplementation is necessary. In horse studs, colostral IgG concentrations are usually evaluated immediately after foaling (Slovis and Vaala 2011), by measuring TPs with a refractometer (McCue 2014) and this practice could be usefully adopted also for donkeys.

Moreover, for this reason, it would be essential to establish cut-offs regarding the quantity of colostral and neonatal γ-globulins suitable for the species, since, according to this study and that of Veronesi et al. (Veronesi et al. 2014), it seems that γ-globulin concentrations in the foals' sera which are considered low for horses are not so low for donkeys. In any case, it is also advisable for a donkey farmer to create a colostrum bank with the best quality colostra to thaw and

administer orally if needed (Vivrette 2011; Turini, Nocera, et al. 2020b).

Conclusion

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.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical approval

The work was approved by the Ethical Committee of the Department of Veterinary Sciences of the University of Turin, Italy (protocol number 311/21). All procedures were performed in compliance with the guidelines of the Italian Ministry of Health for the care and use of animals (D.L. 4 March 2014 n. 26 and D.L. 27 January 1992 n. 116) and with EU Directive 86/609/CEE.

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Data availability statement

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

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