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

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

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8

9   **Abstract**

10   An inadequate colostrum intake results in Failure of Passive Transfer, a condition that makes

11   foals more susceptible to potentially fatal infectious diseases. The aim of the study was to

12   evaluate the transfer of passive immunity in the donkey, using electrophoresis as main

13   diagnostic tool. A group of 20 Ragusana crossbreed jennies (age 3-19 years) and their foals

14   were enrolled. The γ-globulin content of colostrum and dams' and foals' sera was measured,

15   then the effects of foals' season of birth and age and parity of the jennies on γ-globulin

16   concentration and on the efficiency of the immune transfer were evaluated. Influence of

17   *season* factor was analysed by grouping the data on the basis of foaling season (spring,

18   summer or autumn). For the evaluation of *age* and *parity* the jennies were divided into two

19   categories: younger/older and primiparous/pluriparous, respectively. Finally, the possible

20   association of these factors with the efficiency of the immune transfer was investigated.

21   According to the horse reference range, 70% of donkey foals showed complete transfer of

22   passive immunity (γ-globulin>8 g/L; 13.15±4.60 g/L) and 30% had a partial Failure of

23   Passive Transfer (γ-globulin 4-8 g/L; 5.78±1.29 g/L), but without showing clinical signs.

24   Age and parity did not significantly affect passive immunity transfer, nor did the season.

25   Total Protein values measured through refractometer were positively correlated to the γ-

26 globulin content (r=0.69; p<0.01), confirming the possibility to use this diagnostic tool in the

27 field as a first, inexpensive approach for colostrum evaluation.

28

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

30

31 **Highlights**

32   1. The transfer of passive immunity in the donkey is still poorly investigated.

33   2. We investigated the transfer of passive immunity in donkeys using electrophoresis.

34   3. Influence of age, parity and season on the immune transfer has been evaluated.

35

36 **1. Introduction**

37 Although the global number of donkeys appears to be steadily decreasing (Bough 2011), the

38 demand for donkey milk is increasing all over Europe due to its nutritional and cosmetic

39 properties (Dai et al. 2019). The survival of each and every donkey foal is crucial for

40 ensuring sufficient milk production, owing to the uniparity of the species and the long

41 gestation period (372-374 days) (Wilborn and Pugh 2011; Carluccio et al. 2015).

42 After birth, the foal's immune system is immature and does not guarantee adequate

43 protection, as it lacks circulating antibodies which do not cross the epitheliochorial placenta

44 (Bernard and Barr 2012). The colostrum, taken during the first 24 h after birth, provides

45 immunity to the foal for the first few weeks of life and its high protein content reflects the

46 concentration of immunoglobulins (Ig) produced in late pregnancy. Of the four types of Ig

47 (IgA, IgM, IgE and IgG), the IgG, represented by the γ-globulin family, are particularly

48 important in foals (Perkins and Wagner 2015). In the horse, the intestinal absorption of Ig

49 reaches its peak soon after birth, then it begins to drop, decreasing to 28% from 12 to 18

50 hours after birth (McKenzie 2018). This drastic fall is due to the replacement of specialized

2

51 enterocytes with mature ones, unable to absorb large proteins, which takes place regardless of

52 whether or not colostrum intake has occurred and antibodies have been successfully absorbed

53 (Bernard and Barr 2012). The first feeding is the one that provides the maximum Ig

54 concentration, which is destined to rapidly decrease (Brinsko et al. 2010). As a consequence,

55 the foal should receive colostrum within the first 2 hours after birth (Jeffcott 1972; Vivrette

56 2011). Within 4-6 hours after ingestion, Ig enter the foal's bloodstream, peak 24-48 hours

57 post parturition and subsequently decline due to protein catabolism and plasma volume

58 expansion (Bernard and Barr 2012). Initially, serum Ig concentrations in the foal reflect the

59 maternal ones, then progressively decrease as a result of their utilization (Perkins and Wagner

60 2015).

61 The process of intestinal absorption and the use of colostral antibodies by the foal is called

62 'transfer of passive immunity' and any obstacle results in an inadequate concentration of

63 serum Ig in the newborn, called 'Failure of Passive Transfer' (FPT) (Jeffcott 1972). A close

64 relationship has been demonstrated between low serum IgG concentration in foals and the

65 incidence of neonatal diseases (Vivrette 2011). In the horse foal, FPT is a widely studied

66 condition associated with IgG serum concentrations under 4 g/L, 12 hours after birth, while

67 regarding the donkey, the knowledge is currently very limited (Veronesi et al. 2014; Turini et

68 al. 2020a, b) and, at present, no rapid tests are available for the specific measurement of Ig in

69 this species.

70 Even though Radioimmunoassay is the gold standard for quantifying serum Ig (IgG-RID),

71 semi-quantitative field tests are the most widely used as rapid diagnostic methods in horses

72 (Lester 2011; Vivrette 2011; Kummer et al. 2018; McKenzie 2018). A positive correlation

73 has been demonstrated between the IgG measured by electrophoresis (EGG, Electrophoretic

74 Gamma Globulins) and the IgG-RID. Thus, the use of electrophoresis to predict FPT has

75 been tested to demonstrate its field suitability (Tscheschlok et al. 2017).

76 This study is aimed to investigate the transfer of passive immunity in the donkey foal, using

77 electrophoresis as the main diagnostic tool to evaluate the IgG in serum and colostrum.

78 Furthermore, the possible influence of age and parity of the jennies and season of the year on

79 the immune transfer has also been evaluated.

80

81 **2. Materials and Methods**

82 *2.1 Animals and clinical records*

83 The Ethical Committee of the University of Turin (Commissione di Etica e Benessere

84 Animale del Dipartimento di Scienze Veterinarie di Torino) approved the study with protocol

85 number 311/21. Twenty Ragusana crossbreed jennies and their 20 newborn foals, housed on

86 a farm intended for the production of organic milk for human consumption and cosmetics,

87 were enrolled in the study.

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

89 - *season*, classifying the jennies into three groups according to the date of delivery (spring,

90 summer and autumn);

91 - *age*, dividing the animals into 'younger' (up to 10 years) and 'older' (over 10 years);

92 - *parity*, separating the jennies into primiparous and pluriparous.

93 It should be clarified that 'younger' and 'primiparous' jennies may overlap in part because

94 this research has been conducted on animals kept in a farm which breeds donkeys for

95 commercial purposes (milk production for human consumption). Therefore, in order to

96 maintain productivity levels compatible with the commercial activity, there are no animals

97 over 10 years of age that are primiparous since the jennies, resulting from internal

98 replacement, start breeding at 3-4 years.

99 The mean age was 9.5±4.9 years (±Standard Deviation; median: 8 years; range: 3-19 years;

100 mode: 8 years). Sixteen jennies (80%) were pluriparous and four (20%) were primiparous

101 (mode: pluriparous). Thirteen (65%) were under 10 years of age, 7 (35%) were 10 years old

102 or older (Table 1) *[Table 1 near here]*.

103 The jennies had been subjected to natural assisted mating on alternate days, based on the

104 behavioural signs of oestrus identified visually and confirmed by ultrasound examination.

105 The pregnancies were monitored by weekly ultrasound, from 14 to 35 days, and monthly up

106 to the 12th month. Data on the pregnancy duration, time of delivery and jennies' health

107 conditions in the days before and after parturition were collected. To determine the term of

108 pregnancy, the size of the mammary gland, milk secretion, the appearance of the external

109 genitalia, the position of the tail, the abdominal profile and, in general, the attitude of the

110 animals were evaluated. The clinical data were supported by the farm calendar in which the

111 expected date of delivery for each donkey, calculated on the basis of the stable average (370

112 days) from the date of the last mating, was reported. In the days immediately preceding and

113 following parturition, the jennies were kept in a delivery room, where a 24/24h monitoring

114 with a wireless camera was performed.

115 In the immediate post-partum period, a Basic Physical Examination (BPE) of the foals, the

116 disinfection of the umbilical cord and verification of colostrum intake were performed. Foals'

117 BPE was repeated daily for the first week post-partum.

118

119 *2.2. Blood and mammary secretion sampling*

120 One colostrum and one blood sample from each jenny, and one blood sample from each

121 newborn, for a total of 60 samples, were collected (20 colostrum samples; 20 jennies' blood

122 serum samples; 20 foals' blood serum samples).

123 Milking was performed manually within 1 hour after foaling and always before the foal got

124 up for the first feed. At least 5 mL of colostrum were milked from both nipples, previously

125 cleaned, and collected in a 50 mL Falcon tube, after eliminating the first drops. The sample

126 was then split into multiple 1.5 mL Eppendorf tubes.

127 Twenty-four hours post-partum, a blood sample was collected (9 mL) from each jenny and

128 foal from the jugular vein, using a Vacutainer® tube. The serum was obtained by

129 centrifugation (1,000 g for 10') and divided into several 1.5 mL Eppendorf tubes.

130 All samples were identified and immediately refrigerated, then frozen at -20° C until

131 analysis, carried out in the laboratory of the University Veterinary Hospital (OVU) of the

132 University of Turin (Italy).

133

134 *2.3 Total Proteins and γ-globulins Analysis*

135 The total proteins (TP) contained in each sample were determined using a hand-held Reichert

136 Vet 360 optical refractometer (Reichert Technologies, Buffalo, New York, USA), in

137 accordance with Elsohaby et al. (2018), who demonstrated that in horse foals the serum TP

138 concentration measured with this technique is positively correlated with the RID-Ig.

139 Hydrasys (Sebia Italia S.r.l., Bagno a Ripoli, Florence, Italy), a semi-automatic

140 multiparametric instrument, with Hydragel Protein(E) 15/30 agarose gel was used to quantify

141 colostral and serum Ig. The instrument automatically performed the electrophoretic

142 migration, washing, drying and colouring of the gel that was placed in the scanner for the

143 densitometric reading of the protidogram thereafter. The relative concentration of each

144 protein fraction was interpreted as a percentage of the optical absorption, based on the

145 absolute concentration (g/L) of the TP of the sample. The electrophoretic curves were read

146 and possibly corrected using the Phoresis software (Sebia Electrophoresis®, Sebia Italia S.r.l.,

147 Bagno a Ripoli, Florence, Italy). The percentage and g/L values of Electrophoretic Gamma

148 Globulins (EGG) were obtained.

149

150 *2.4 Statistical Analysis*

151 All data underwent descriptive statistics.

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

153 The differences in pregnancy length and foals' weight in relation to the sex of the foetus were

154 analysed by means of independent sample t-test, while a possible relationship between

155 pregnancy length and foals' weight was investigated with Pearson correlation.

156 The mean, standard deviation, median and range of total protein (TP) and γ-globulin

157 concentration in jennies' and newborns' serum and colostrum were calculated.

158 The presence of a possible correlation between TP and γ-globulin concentrations and

159 between the TP as well as the γ-globulin content in the different matrices (foals' and jennies'

160 sera and colostrum) were investigated with Pearson's or Spearman's tests according to the

161 data distribution.

162 The different factors (*season*, *age* and *parity*) that could have influenced the serum and

163 colostral TP and γ-globulin concentrations were analysed.

164 The analysis of variance or the Kruskal Wallis test were performed to investigate the

165 differences between the TP and γ-globulin levels over the seasons.

166 Similarly, to compare the concentrations of TP and γ-globulin in relation to age and parity,

167 independent sample t-test or the Mann–Whitney U test were applied.

168 A possible association between each factor described above and the quality of the colostrum

169 as well as the efficiency of the transfer of passive immunity to the foal were investigated. For

170 this analysis, the samples were divided into groups according to the cut-off values established

171 for the mare (Cash 1999; Tscheschlok et al. 2017). More in details, the colostrum samples

172 have been divided into 4 groups, based on the γ-globulin concentration (Cash 1999): 'very

173 good' quality (IgG > 80 g/L), 'good' quality (IgG between 50 and 80 g/L), 'fair' quality (IgG

174 between 28 and 50 g/L) and 'poor' quality colostra (IgG < 28 g/L). The transfer of passive

7

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).

8

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*

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

225 The levels of γ-globulin in the jennies' sera did not change significantly in the different

226 seasons examined (spring: 17.34±2.27 g/L, summer: 18.07±2.04 g/L, autumn: 21.95±6.53

227 g/L), nor on the basis of age (younger: 18.65±3.62 g/L, older: 18.39±3.99 g/L), or parity

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

229

230 *3.3.2 Concentration of γ-globulin in foals' sera*

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

232 Season of birth (spring: 11.07±5.94 g/L, summer: 10.16±2.69 g/L, autumn: 12.22±8.21 g/L),

233 jennies' age (younger: 10.71±5.05 g/L, older: 11.37±5.83 g/L) and parity (primiparous:

234 8.90±3.48 g/L, pluriparous: 11.45±5.51 g/L) did not significantly affect serum γ-globulin

235 concentration in newborns.

236 Based on the classification established for the horse (Tscheschlok et al. 2017), 14 of 20

237 donkey foals (70%) had received a complete transfer of the passive immunity, with

238 concentrations of γ-globulin > 8 g/L (mean: 13.15±4.60 g/L; median: 11.15 g/L; mode: 5.00-

239 12.00 g/L; range: 8.30-23.70 g/L). The other 6 foals (30%) had a PFPT (mean γ-globulin

240 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).

241 FPT (γ-globulin < 4 g/L) was not found in any newborn.

242 Season of birth, age and parity of the jennies were not statistically associated with the

243 effectiveness of the transfer, either complete or partial, even if a lower incidence of PFTP has

244 been observed in summer, although in the absence of statistical evidence.

245

246 *3.3.3 γ-globulin in colostrum*

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

248 Concentration of γ-globulin in the colostrum did not vary significantly in relation to the age

249 (younger: 79.85±29.71 g/L, older: 57.22±16.13 g/L) and parity (primiparous: 76.22±22.78

250 g/L, pluriparous: 70.86±29.25 g/L) of the jennies nor in relation to the season (spring:

251 58.78±17.12 g/L, summer: 83.21±19.87 g/L, autumn: 75.67±49.02 g/L).

252 Classification of colostrum samples according to γ-globulin content, on the basis of the

253 qualitative categories established for the mare (Cash 1999), showed a majority of high-

254 quality samples: 6 colostra (30%) were of 'very good' quality (IgG > 80 g/L; average:

255 105.33±22.78 g/L, median: 100.22 g/L, mode 80-110 g/L, range: 84.59-146.75 g/L), 11

256 colostra (55%) were of 'good' quality (IgG between 50 and 80 g/L; mean: 62.74±9.73 g/L,

257 median: 56.50 g/L, mode: 50.00-60.00 g/L, range: 52.40-79.90 g/L), 3 colostra (15%) were of

258 'fair' quality (IgG between 28 and 50 g/L; mean: 38.82 ± 3.91 g/L, median: 38.20 g, mode:

259 34.00-39.00 g/L, range: 35.26-43.00 g/L). No 'poor' quality colostra have been found (IgG <

260 28 g/L).

261 Colostrum quality (very good, good or fair) was significantly associated with the efficiency

262 of the transfer of passive immunity (complete or PFPT) ($p<0.05$). However, considering the

263 quality of the colostra in the examined seasons, the good quality colostra were distributed in a

264 similar way in spring and summer, but among the 6 colostra containing more than 80 g/L of

265 γ-globulin, 4 were collected in summer (66.6%), 1 in spring (16.7%) and 1 in autumn

266 (16.7%).

267

268 *3.3.4 Correlation between γ-globulin in the different samples*

269 There was no significant correlation between jennies' serum and colostrum γ-globulin

270 concentration (ρ=0.29), nor between the jennies' and newborns' sera (ρ=0.05), but a positive

271 and statistically significant correlation has been found between the γ-globulin concentration

272 in the foal's serum and colostrum (r=0.53, $p<0.05$; Table 3).

273

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).

321   To date, few papers have been published on IgG serum concentration of the donkey foal

322   (Veronesi et al. 2014; Turini et al. 2020a). Our values are slightly higher (10.94±5.19 g/L)

323   than those observed by Veronesi et al. (2014) (8 g/L, 12 h after birth), but in line with those

324   reported by Turini et al. (2020a) (14.91±0.50 g/L, 24 h after birth). The difference among the

325   studies could be due to several reasons: our sample size was larger and the diagnostic method

326   was different. In this research jennies were crossbred, while the other studies referred to

327   purebred animals, Martina Franca (Veronesi et al. 2014) and Amiata (Turini et al. 2020a).

328   Finally, as reported by Turini et al. (2020a), the fact that in Veronesi's et al. work (Veronesi

329   et al. 2014) the jennies had been milked in the days before giving birth, could have slightly

330   influenced the post-foaling colostral quality.

331   In our work, 30% of the foals had PFPT, based on the classification established for the horse

332   (Tscheschlok et al. 2017), however all were apparently healthy in the days following

333   parturition, showing normal neonatal development. We did not observe FPT and even foals

334   with a very low IgG content (IgG < 1.8 g/L) at 24-48 hours showed no signs of pathology, as

335   reported by Veronesi et al. (2014). The association between a very low γ-globulin

336   concentration and the absence of clinically evident neonatal diseases is extremely anomalous.

337   The hypothesis that can be formulated is that the minimum antibody coverage that the

338   donkey foal requires in the first days of life is lower than that needed by the horse foal, also

339   because, in donkeys, the non-specific immunity provided by lysozyme, very abundant in

340   colostrum and in donkey milk, seems to play a key role (Qureshi and Enbergs 2012; Veronesi

341   et al. 2014). It would be interesting to evaluate serum IgG concentration of pathological foals

342   to understand if the cut-off for defining the failure of passive immunity transfer in the donkey

343   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).

420 **5. Conclusions**

421 The transfer of passive immunity from jenny to foal is still largely unknown and, currently,

422 there are no in-depth studies on the factors that can influence this delicate immune function.

423 Apparently, this process is similar in horses and donkeys. However, it would be interesting to

424 investigate the relationship between the donkey's seasonality and its reproductive activity,

425 referring to a larger population of animals. Comparing the donkey to the horse there is, in

426 fact, the risk of not grasping the subtle differences that make the breeding of these two

427 species completely different.

428

429 **Declarations of interest statements**

430 The authors declare no conflict of interest.

431

432 **Data availability statement**

433 The data that support the findings of this study are available from the corresponding author,

434 [A.B.], upon reasonable request.

435

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