Original Research

Journal of Advanced Veterinary Research
(2025) Volume 15, Issue 4, 431-435

Association of passive transfer failure of colostrum and serum immunoglobulins with risk factors in both natural and embryo transfer arabian foals

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ARTICLE INFO

Recieved: 27 May 2025

Accepted: 25 July 2025

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Keywords:

Natural foals, Embryo transfer foals, Risk factors, Immunoglobulins, Refractometer, Zinc sulphate.

ABSTRACT

Thirty Arabian foals were included in this study, 15 produced by natural breeding and the others by the embryo transfer technique. This study was conducted during the first 24 hours of the foal's life, as the foal immunoglobulin (IgG) levels were measured using three different methods. The first method, the most reliable in this study, was the ELISA test (reference method). The other methods used for serum analysis were the refractometer and zinc sulfate test, while the colostrum immunoglobulins (IgG) of mares were measured only by refractometer. Furthermore, some statistical analyses were performed to assess the diagnostic test characteristics of the refractometer and zinc sulfate test, which were used to calculate the optimal cut-off values for estimating different passive immunity statuses in foals. The optimal cut-off values were selected based on sensitivity and specificity, as the best cut-off point contains the ideal combination of these factors. Additionally, the calculation of the area under the curve (AUC) for the refractometer and zinc sulfate tests provided information about the accuracy of the tests in differentiating foals with and without failure of passive transfer (FPT). This study aimed to estimate the risk factors associated with the failure of passive transfer. The results revealed that these factors are related to the dam, including age, parity, pregnancy type (whether broodmare or recipient), body weight, body condition scores, gestation period, mineral salt administration, and measurement of colostrum IgG by refractometer, as well as serum IgG by ELISA, zinc sulfate, and refractometer. On the other hand, factors related to foals included birth weight, number of navel disinfection, foaling time, gender, and measurement of IgG in serum by ELISA, zinc sulfate, and refractometer.

Introduction

Foals are mostly born agammaglobulinemic and acquire their immunity through the absorption of colostrum, as the horse placenta is diffuse epitheliochorionic, which does not permit the transmission of antibodies from the dam to the fetus during the gestation period (Mackenzie, 2020). Specialized cells in the mucosa of the small intestine of newborn foals absorb immunoglobulins through pinocytosis. These cells are replaced by more mature cells within 24 hours, resulting in "gut closure" (Aoki *et al*., 2020). Thus, the foals' intestinal mucosa loses its ability to absorb immunoglobulins about 24 hours after birth. Furthermore, consumption of colostrum by normal foals begins within 2 hours after birth, and maternal antibodies are detected in the foals' serum within 4 to 6 hours. Most of the immunoglobulins in colostrum are immunoglobulin G, with small amounts of IgA and IgM (Mehmet *et al*., 2023). Therefore, newborn foals must consume sufficient colostrum within the first 2 months of life to avoid failure of passive transfer (FPT).

Failure of passive transfer is described as the failure to ingest or absorb colostrum by the foal and is considered one of the most important immunodeficiency disorders in newborn foals, with an incidence rate between 3-24%. It occurs due to poor colostrum quality, a small quantity of colostrum, or inadequate intake or absorption of colostrum. Numerous lab-based and lab-independent testing procedures are available to determine the IgG levels in a foal's blood (Maren *et al*., 2022).

Moreover, radial immunodiffusion is considered the gold standard. However, other tests, such as ELISA, are used reliably, along with non-laboratory-based tests like the zinc sulfate test (Maren *et al*., 2022). Foals with FPT are at increased risk of infection or death within the first month of life. If circulating IgG concentrations are less than 400 mg/dL at 24 hours of age, the foal is deemed to have FPT. Blood concentrations of > 800 mg/dL are considered satisfactory transfer of immunoglobulins, whereas 400–800 mg/dL is considered partial FPT (Mackenzie, 2020).

One of the indirect techniques for assessing the transfer of immunoglobulins is the refractometer. It is regarded as a rapid, inexpensive method and is easy to use under field conditions for measuring IgG concentration in colostrum (Elsohaby *et al*., 2015; Denholm *et al*., 2021).

Besides, the quality of colostrum can be determined by its IgG content and classified as follows: "very good" if the IgG concentration is more than 80 mg/mL; "good quality" if the IgG concentration is between 50 and 80 mg/mL; "fair quality" if the IgG concentration is between 28 and 50 mg/mL; and "poor quality" if the IgG concentration is less than 28 mg/mL (Luca *et al*., 2020). The Brix refractometer is considered a screening test for FPT. It is used to measure the concentration of total protein, which allows estimation of immunoglobulin concentration, as other proteins remain constant in a normal foal (Gilvannya *et al*., 2021).

The zinc sulfate turbidity test is an indirect and subjective method for evaluating a foal's immunological status. It is relatively inexpensive and provides quick results, enabling early identification of foals with FPT (Pompermayer *et al*., 2019). This study aimed to determine the risk factors associated with the failure of passive transfer in Arabian foals produced by natural breeding and embryo transfer techniques.

Materials and methods

This study was conducted with the approval of the Veterinary Institutional Animal Care and Use Committee (IACUC vet cu 01122022622) at Cairo University, Egypt. It was performed on thirty Arabian foals, 15 produced by natural breeding at the EL-Zahra Stud Station and the others produced by embryo transfer at private farms between January 2021 and April 2023.

Sampling

Milk samples were collected from thirty mares directly after parturition. The dam's udder was cleaned of debris and bacteria using warm water and soap. The first strip of colostrum was discarded, and 10 mL

ISSN: 2090-6277/2090-6269/

of colostrum was collected in a sterile milk tube from every mare once after parturition . Venous blood samples were collected from 30 foals (all foals suckle normally no artificial feeding) after birth (within 24 hours) and weekly for 2 month then centrifuged at 3000 rpm. The serum was separated for cryopreservation at -20°C.

Techniques for different measuring methods

The ELISA technique was implemented to detect immunoglobulin G (IgG) in foal serum accurately. The sandwich ELISA kit (Horse Immunoglubulins G Eliza Kit – China) was used, with the plate precoated with horse IgG antibodies. The sample (IgG) was added and bound to these antibodies. Then, a biotinylated horse IgG antibody was added and bound to the IgG in the sample, and then streptavidin-HRP was added. The reaction was determined by adding an acidic stop solution, and absorbance was measured at 450 nm.

A refractometer was used for both mare colostrum and foal serum. Milk samples were collected, as mentioned previously, and analyzed twice by the same operator, with the average taken. Using a Brix refractometer (0%–32%), one large drop of colostrum was placed on the prism of the refractometer and covered with a glass lid. In front of a light source, the resulting value was read at the boundary between the light and dark regions. After each sample, the prism was cleaned. Calibration of the refractometer was done prior to use. The results were evaluated according to Luca *et al*. (2020) Serum samples were taken by placing one to two drops on the prism, covering it with the glass lid, and reading the resulting value at the line between the light and dark areas. The same samples were then analyzed in the laboratory using the ELISA technique.

The zinc sulfate turbidity test was utilized to measure the concentration of immunoglobulins in serum. The turbidity is proportional to the concentration of serum immunoglobulins. A barium sulfate solution was used as a turbidity standard, providing 20 units equivalent to 400 mg/dL of IgG. A calibration curve was built by two fold serial dilution of the barium sulfate solution. The turbidity was measured spectrophotometrically at λ = 550 nm (Hogan *et al*., 2016). IgG concentration was calculated using a conversion factor of 20 as follows:

IgG (mg/dL) = Turbidity units × Dilution factor × Conversion factor.

Estimation of risk factors

The data were collected from available records of Arabian mares and categorized based on their association with the dam or foal. Risk factors related to the mare included age, parity (multiparous or primiparous), pregnancy type (broodmare or recipient), body weight, body condition score, gestation period, mineral salt administration, and colostrum IgG measurement using a refractometer only, whereas serum IgG was measured using ELISA, zinc sulfate, and a refractometer.

Potential risk factors associated with foals included birth weight, weight at one month, number of navel disinfections, time of birth (day or night), gender, and serum IgG measurement using ELISA kits in the laboratory as a reference test, a refractometer as a field method, and zinc sulfate as a screening test. In this study, failure of passive transfer (FPT) was determined by the IgG level within the first 24-48 hours. A foal with serum IgG below 4 g/L was classified as having FPT, whereas a level above 4 g/L indicated successful passive transfer of IgG .(Mackenzie, 2020).

Statistical analysis

Using SPSS software (SPSS 24.0; SPSS Inc., Armonk, NY, USA), different statistical tests were applied to the data collected from Arabian mares and foals. Pearson chi-square was used to determine the significance between FPT and risk factors. Relative risk and odds ratio were calculated for the association between chi-square and these factors.

A receiver operator characteristic (ROC) curve was used to evaluate the performance of the refractometer and assess the passive immunity status of Arabian foals concerning the results of the ELISA technique. Diagnostic test characteristics of the refractometer were calculated to determine optimal cut-off values for estimating different passive immunity statuses in foals. The optimal cut-off values were selected based on sensitivity and specificity, with the best cut-off point defined as the one containing the ideal combination of sensitivity and specificity. Sensitivity is defined as the proportion of foals that tested positive for FPT (based on ELISA), and specificity is the proportion of foals that tested negative for FPT (based on ELISA). Calculating the area under the curve (AUC) summarizes the test's ability to differentiate foals with and without FPT. An AUC value > 0.9 indicates high accuracy, 0.7-0.9 indicates moderate accuracy, and 0.5-0.7 indicates low accuracy (Deelen *et al*., 2014).

Results

Thirty foals and thirty mares were included in this study. The measurement of risk factors and relative risk for the dam indicates that foals from multiparous mares are less exposed to failure of passive transfer than those from primiparous mares, and foals from brood mares are less exposed than those from receptor mares (Table 1). An increase in the gestation period of mares (320-340 days) and the administration of mineral salt in the ration positively affect the passive transfer of immunoglobulins. This study found that parturition during daylight has a more negative effect than at night. Our results revealed that the foal's gender impacts FPT, as females receive less exposure than males. Additionally, an increasing number of naval disinfections (> 5 times) influences passive transfer (Table 2).

Based on the ELISA technique (as a reference test), the serum of foals was analyzed, and about 23.3% (7/30) of foals were classified as a failure of passive transfer (IgG < 4 g/L), while 76.6% (23/30) showed passive transfer of immunoglobulins (IgG > 4 g/L). According to the refractometer test, 33.3% (10/30) of foals were classified as FPT (IgG < 7.9 mg/mL), and 66.6% (20/30) were classified as having passive transfer (IgG > 7.9 mg/mL). By employing the zinc sulfate test, half of the foals (50%) were classified as FPT (IgG < 418 mg/dL), and 50% were classified as having passive transfer (IgG > 418 mg/dL).

The ROC curve for the optical refractometer was measured with the ELISA test to detect failure of passive transfer (Figure 1). Sensitivity and specificity were calculated for each cut-off value, with the best cut-off point being 7.9 mg/mL and an AUC of 0.713, indicating moderate accuracy of the optical refractometer compared to the ELISA test (Table 3). When the AUC of the zinc sulfate test was compared to the ELISA test, it was 0.693, and the best cut-off point was 418.0439, indicating that zinc sulfate has low accuracy compared to the ELISA test (Figure 2).

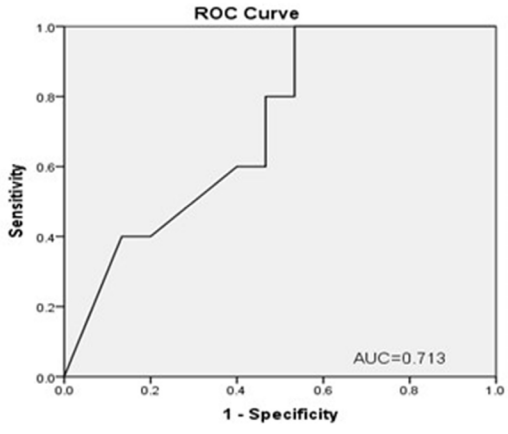

Figure 1. The receiver operating characteristic (ROC) curve for the optical refractometer (Brix %) is used to detect the failure of passive transfer in foals, as measured by ELISA.

Table 1. Chi-square test results assessing the statistical significance of the correlation between various risk factors and FPT

Risk factors	Variable	FPT
Maternal factors		
Parity	Multi or primi	0.39
Pregnancy type	Broodmare or receptor	5.05
Mother age	6-8 y, 8-10 y, >10 y	7.83
Mare weight		18000
Maternal body condition	Poor, thin, moderate, good, Obese	6.67
Gestation period	< 320, 320-340	0.39
Mineral salt administration	Yes or No	3.2
Mare colostrum IgG by refractometer	Scale	15.2
Mare serum IgG by refractometer	Scale	9.87
Mare serum IgG by Eliza	Scale	15.2
Mare serum IgG by zinc sulfate	Scale	20000
Foal factors		
Foal weight	0-40 kg, 50-60 kg, > 60 kg	3.2
Foal weight in the first month	60-80 kg, 80-100 kg, 100-120 kg	5.14
Gender	Male or female	3.33
Time of birth	Day or night	3.2
Number of naval disinfection	Once, 2-5 times, > 5 times	3.2
Foal serum IgG by ELISA	Scale	16000
Foal serum IgG by refractometer	Scale	20
Foal serum IgG by zinc sulfate	Scale	20

Table 2. Relative risk factors and odds ratios that have a significant association with FPT using chi-square analysis.

Risk factors	R.R	Odds ratio
Maternal factors		
Parity	0.81	2.25
Pregnancy type	1.94	9.33
Mineral administration	1.38	5.44
Gestation period	1.45	2.25
Time of birth	1.13	0.29
Foal factors		
Gender	1.17	0.17
Number of naval disinfection	1.38	0.18
Foal weight	1.07	1.5

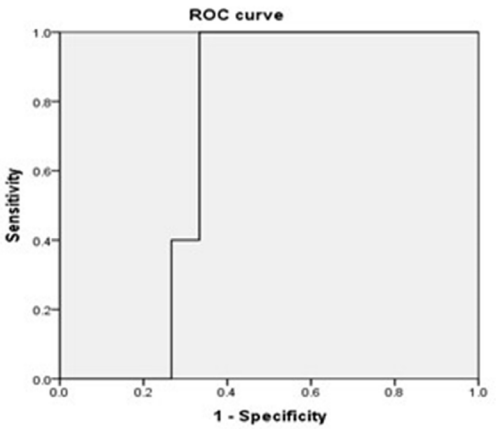

Figure 2. The receiver operating characteristic (ROC) curve for zinc sulfate was measured by ELISA to detect failure of passive transfer in foals.

Discussion

Failure of passive transfer (FPT) is described as the inability of the foal to ingest or absorb sufficient quantities of immunoglobulins, and it is the most significant risk factor for the development of infection and death within the first month of life (Sellon, 2000)

In this study, FPT depended on multiple risk factors associated with the dam and the foal. Foals born to multiparous mares were less exposed to FPT than those born to primiparous mares (0.8125 times lower risk). This result is consistent with the findings of Franco Ayala and Oliver-Espinosa (2016) and Shideler *et al.* (1986) but contrasts with a study conducted on standardbred foals, which found that primiparous mares exhibited lower FPT in their foals, although this difference was not statistically significant. According to Clabough *et al.* (1991), the number of pregnancies affects the level of immunoglobulins. Conversely, research by Kenzig *et al.* (2009) and Morris *et al.* (1985) found that the number of foalings (parity) did not significantly affect immunoglobulin levels and, consequently, the occurrence of FPT.

Additionally, our study found that female foals had higher concentrations of IgG than male foals (1.66 times higher). This finding is consistent with the results of Erhard *et al.* (2001) and Jeffcott (1972). Some studies have shown that the gestation period does not significantly affect FPT, our study demonstrated that the gestation period had a significant effect (1.445 times higher risk). Furthermore, mineral salt administration and foaling at night were found to have a positive effect on reducing FPT risk, while the number of naval disinfectations was associated with a lower risk of FPT (1.381 times). This finding contradicts the study by Sellon (2000); Erhard *et al.* (2001) and Jeffcott (1972).

Lastly, our study showed that foal weight and pregnancy type (broodmare versus receptor mare) had a significant effect on FPT (1.0715 and 1.9375 times, respectively) (Table 2). There is limited research on these factors in the literature.

Newborn foals must consume colostrum (Robinson *et al.*, 1993), as a lack of it can lead to neonatal septicemia (Sanchez, 2005) and a high mortality rate (Hass & Card, 1996). However, in the present study, the quality of mare colostrum was not significantly associated with FPT, which is consistent with the findings of Sellon (2000).

A sandwich ELISA system was employed to measure foal IgG antibodies and determine FPT. The ELISA test is a reliable method that is sensitive and specific for diagnosing FPT (Pusterla, *et al*, 2002; Davis and Giguère, 2005), as it is reproducible and easier to perform than other

Table 3. The optical refractometer and zinc sulfate characteristics for detecting FPT, determined by the ELISA technique as the reference test, including cut-off points, sensitivity, and specificity.

Cut-off point g\dl	Sensitivity (%)	1-Specificity (%)
Optical refractometer		
5	0	0
6.4	0.2	0.07
6.85	0.4	0.13
7.05	0.4	0.2
7.3	0.6	0.4
7.45	0.6	0.47
7.55	0.8	0.47
7.7	0.8	0.53
7.9	1	0.53
8.15	1	0.6
8.35	1	0.67
8.5	1	0.73
8.75	1	0.8
9.05	1	0.87
9.4	1	0.93
10.6	1	1
Zinc sulfate		
203.17	0	0
210.65	0	0.07
256.03	0	0.13
306.70	0	0.2
318.49	0	0.27
319.45	0.2	0.27
339.48	0.4	0.27
360.33	0.4	0.33
380.85	0.6	0.33
400.85	0.8	0.33
418.04	1	0.33
438.11	1	0.4
445.77	1	0.47
466.40	1	0.53
487.61	1	0.6
504.70	1	0.67
523.56	1	0.73
584.84	1	0.8
647.90	1	0.87
667.93	1	0.93
681.31	1	1

methods (McGuire & Crawford , 1973) . A comparison between the ELISA test and refractometer revealed an AUC of 0.713, indicating that the refractometer has moderate accuracy in differentiating foals with and without FPT. This result agrees with the study by Maren *et al*. (2022), who reported an AUC of 0.794, while Elsohaby *et al*. (2019) found an AUC of 0.83. Some authors have suggested that the refractometer is unsuitable for measuring serum IgG levels below 4 g/L and should only be used to diagnose FPT under limited conditions. However, others argue that the refractometer can still be acceptable if a higher cut-off value is used, leading to higher sensitivity and lower specificity (Elsohaby,*et al*., 2019; Metzger *et al*., 2006).

In contrast, the comparison between ELISA and zinc sulfate revealed an AUC of 0.6, indicating that zinc sulfate has low accuracy in differentiating foals with FPT. This finding has not been reported in previous research. From this study's perspective, the refractometer and zinc sulfate can be used as simple, inexpensive, and rapid field tests for initial diagnosis on farms, with the refractometer proving to be more accurate than zinc sulfate. However, further studies are needed to examine the risk factors and the use of refractometer and zinc sulfate with a larger sample size.

Conclusion

Foals from brood mares have a lower risk of FPT than those from receptor mares (embryo transfer). Additionally, gestation period, foal weight, and the time of birth (day or night) affect significantly the passive transfer of immunoglobulins. Foals born from multiparous mares are less likely to experience FPT than those born from primiparous mares. Furthermore, the refractometer has moderate accuracy in diagnosing FPT, whereas zinc sulfate demonstrates low accuracy compared to ELISA as the reference test.

Acknowledgments

The authors thank Alzahraa Arab Stud. for graciously allowing the use of their farm as a study site.

Conflict of interest

The authors have no conflict of interest to declare.

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