

# Comparison of Serum IgG Concentration, Total Protein, Glutaraldehyde Coagulation Test and Gamma Glutamyl Transferase in Neonatal Foals

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## Abstract

The purpose of the present study was to detect the passive transfer status in healthy neonatal foals by comparing serum immunoglobulin G (sIgG) concentration, serum total protein (STP), glutaraldehyde coagulation test (GCT) duration and gamma glutamyl transferase (GGT) activity. Fifteen neonatal foals (0-15 days old) blood samples were collected before suckling (day 0), 12th hour (hr), 24th hr (1st day), 7th and 15th days. Serum IgG and STP levels significantly increased after the 12th hr. Conversely, serum GCT duration significantly decreased ( $p < 0.05$ ) in neonatal foals after the 12th hr. The result of the present study was shown that measurements of sIgG, STP concentration and GCT duration are useful parameters to detect Failure of Passive Transfer (FPT) in neonatal foals. While GCT and STP provide a simple and inexpensive field test, serum GGT measurement is not a beneficial test to determine colostrum intake in newborn foals.

**Key Words:** Foals, Newborn, Failure of Passive Transfer, serum Immunoglobulin G

## Introduction

Foals are born without appreciable serum concentrations of immunoglobulin (Ig). Colostral Ig is required to supply humoral immunity during the neonatal period. Immunoglobulin requirements are satisfied by ingesting and absorbing colostrum Igs. The absorption of Igs from colostrum is a vital importance for foals' health. Failure of passive transfer was increased the rate of illness and death during 6-7 weeks of age.<sup>1,2</sup> The majority of Ig in equine colostrum is serum immunoglobulin G (IgG), which provides protection against infection for the first one to two months of life.<sup>3</sup> One of the most important causes of infectious diseases (septicemia, enteritis, pneumonia, arthritis etc.) are complete (sIgG:0-400 mg/dl) or partial (sIgG: 401-800 mg/dl) FPT in the neonatal period of foals.<sup>1</sup> The major cause of neonatal death is septicemia in the first week of their life.<sup>4,5</sup> Many studies have shown that FPT occurs in

10-20% of foals and rises to 30% in those animals that require hospitalization.<sup>3</sup>

There are several methods for determining of sIgG levels in foals. Diagnostic tests include single radial immunodiffusion<sup>6</sup>, GCT,<sup>1,7</sup> zinc sulfate turbidity, <sup>8</sup> latex agglutination,<sup>1</sup> Enzyme Linked Immunosorbent Assay,<sup>9,10</sup> turbidimetric immunoassay,<sup>3,6</sup> determination of STP,<sup>6,10</sup> and serum globulin concentration.<sup>10</sup> Glutaraldehyde coagulation test (GCT), which is introduced for detection of hypergammaglobulinaemia using whole blood, is a semiquantitative, practical, inexpensive, fast and especially amenable to field conditions. Serum total protein (STP) measurement is an alternative method for detecting the concentration of IgG for diagnosing FPT in foals and it can be measured in the laboratory but may also be approximated using a refractometer.<sup>6,11</sup> In decade, GGT is the most popular diagnostic method to diagnose of the FPT in cattle. However, there

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are limited scientific knowledge about importance in diagnosis of FPT in foals.

The aims of this study were to determine the suitability of the GCT, STP, and GGT activity based on total IgG in foal blood.

## Materials and Methods

### Study Design

In this study, 15 foals (0-15 days) born on a breeding farm located at Karacabey, Bursa, Turkey. All foals and their mothers were clinically examined daily until 1 month of age after birth, and they were healthy. All applications were performed under the control and approval of the University of Ethical Committee, in accordance with the Animal Welfare Guidelines (2014-10/03). The blood samples were collected from the jugular vein using plain vacuum tubes at before suckling (day baseline), after 12th hr, 1st (24th hr), 7th and 15th day of the study. Blood samples were centrifuged and then serum samples were stored at -20° C until analysis.

### Total IgG, TP, GCT, and GGT measurement

The concentrations of sIgG were measured by an immunoturbidimetric method (MBC QTII®)<sup>3,6</sup>. The range of measurement is 200 to 1700 mg/dl. This method is a linear representation of Equine IgG levels until 1700 mg/dl. Samples higher than 1700 mg/dl were re-assayed after dilution with 0.9% saline. Serum GGT activity was analyzed by an en-

zymatic colorimetric test (Roche Cobas Integra 400 Plus). Serum GCT duration was measured by using a 10% glutaraldehyde solution (Merck). 0,5 ml of each serum sample were mixed with 50 µl solution into the test tubes and examined for coagulation during 1 hour. A positive coagulation reaction was said to occur when a solid gel. Serum TP was measured by a hand refractometer. After calibration, a few sample drops were placed on the surface of the prism. The daylight plate was closed gently and the protein scale was read.

### Statistical analyzes

The data were analyzed using the Friedman test, a non-parametric test, because the data was not passed the normality test (SPSS, windows version 13.0). Pearson's correlation analysis was calculated between sIgG concentrations, STP activity, GCT duration and GGT activity. The level of significance was set at P<0.05.

## Results

The results about IgG concentrations, STP levels, GCT duration, and GGT activities are summarized in Table 1. Serum IgG levels were too low before suckling in this study. It is important to note that the commercial kit (MBC QTII®) did not provide IgG results measuring less than 200 mg/dl, so the mean for this measurement is not entirely accurate. The correlations were indicated between sIgG concentrations, GGT activities, GCT duration and STP activities in

Table 1. Serum IgG, STP, GCT, and GGT findings (mean±SE) in newborn foals (n:15)

	<u>Presuckling</u> (n:15)	12hour (n:15)	1st day (n:15)	7th day (n:15)	15th day (n:15)
IgG (mg/kg)	<200 <sup>b,c,d,e</sup>	1215.06±151,3 <u>a,c,d,e</u>	1643.9±64 <sup>a,b,e</sup>	1561.9±75 <u>a,b</u>	1491.2±84.96 <u>a,b,c</u>
GGT (IU)	13.62±4.7 <sup>c,d,e</sup>	14.527±1.36 <sup>c,d,e</sup>	9.697±0.94 <sup>a,b,d,e</sup>	19.952±6.9 <sup>a,b,c,e</sup>	24.6±6.7 <sup>a,b,c,d</sup>
GCT (minutes)	50±00 <sup>b,c,d,e</sup>	18.933±6.4 <sup>a,c,d,e</sup>	6.467±1.8 <sup>a,b,e</sup>	6.7±1.6 <sup>a,b,e</sup>	4.66±0.37 <sup>a,b,c,d</sup>
(STP ) (g/dl)	3.89±0.12 <sup>b,c,d,e</sup>	7.2±0.2 <sup>a</sup>	7.4±0.1 <sup>a</sup>	7.547±0.13 <sup>a</sup>	7.56±0.1 <sup>a</sup>

a, b, c, d, e are referred to value belong to each column and indicated statistically significance between rows, P<0.05.

Table 2: Correlations between serum IgG concentrations, STP levels, GCT durations, and GGT activities.

	12 hour	1st day	7th day	15th day
IgG and GGT	0.141	-0.206	0.146	0.156
IgG and GCT	-0.729*	-0.901*	-0.955*	-0.288
IgG and STP	0,830*	0.732*	0.575*	0.610*
GGT and GCT	-0.291	-0.0456	-0.229	-0.373
GGT and STP	0.229	-0.148	0.496	0.358
GCT and STP	-0.689*	-0.778*	-0.727*	-0.332

\* $p < 0.05$  represents correlation between the different two variables on same period that are found to be statistically significant.

## Discussion and Conclusion

Measurement of serum IgG levels are very important for detecting an early diagnosis and treatment of FPT. Maximum ingestion of colostrum IgG consists by 12 hours after birth. So, it has been recommended that a neonatal foal's serum should be measured 8 to 12 hours after birth to assess passive transfer status.<sup>12</sup> In this study, serum IgG concentrations were detected as  $1215.06 \pm 151.3$  mg/dl at 12 hours after birth, and maximum concentrations were reached  $1643.93 \pm 64.48$  mg/dl in 24 hours. The absorption of Igs decreases 24 to 36 hours after birth because the specialized enterocytes are replaced by epithelial cells with pinocytosis deficiency.<sup>2,13</sup> The first 24-hours sIgG results of this study are similar findings to other studies (Table 1).<sup>2,13</sup> In line with these results, it may be possible to assess serum IgG levels as a gold standard in relation to previous studies. Early diagnosis of FPT is very important in decreasing the rate of disease in foals. Several studies have suggested that a STP concentration less than or equal to 4.5 g/dL was suggestive of FPT, whereas values greater than or equal to 6.0 g/dL indicated adequate IgG concentrations and that serum TP concentration can be used as an alternative to potentially assess the IgG concentration if no other test can be performed.<sup>6,14</sup>

In this study, STP concentrations were measured as  $3.89 \pm 0.122$  g/dl before suckling and the concentrations increased at 12 h ( $7.227 \pm 0.21$  g/dl) after suckling. This demonstrates that the concentrations of STP increased after 12 hours, as did sIgG concentrations. The major advantages of this test are that it does not contain very special procedures for STP measurement and can be applied in a

farm or a field condition.<sup>11</sup> Also, in this study, a positive correlation was detected between sIgG concentrations and STP levels ( $p < 0.05$ ), and a negative correlation was found between serum TP and GCT duration ( $p < 0.05$ ) at the following time points: 12 hours after birth, and the 1st, 7th and 15th days of the experiment as shown in Table 2. Serum GCT duration is a useful test to evaluate passive transfer status of calves, goat kids and foals.<sup>15</sup> The effect of GCT duration in detecting failure to acquire colostrum immunoglobulin in neonatal foals was investigated by comparing and correlating results from the GCT with those obtained by equine IgG. The GCT duration was found to be a practical, inexpensive, semi-quantitative test with a high specificity and sensitivity at critical IgG levels.<sup>7,16</sup>

In this study, the foals' serum did not clot until reaching 50 minutes in GCT duration before colostrum feeding indicated hypogammaglobulinemia. There was found a negative relationship between the GCT duration and sIgG concentration in the present study. The GCT duration decreased, while serum IgG concentrations and STP activities increased at 12 hours after birth (Table 1). A negative correlation was detected between the GCT duration and sIgG concentrations ( $p < 0.05$ ) from 12 hours after birth, and the 1st, 7th and 15th days of the experiment as shown in Table 2. Hurcombe et al. (2012)<sup>5</sup> proposed that STP levels can be used to estimate the sIgG concentrations in neonatal foals. Moreover, Beetson et al.<sup>5</sup> determined that it is possible to identify foals which have successful colostrum transfer by using the GCT. Although we could not find any study STP and GCT are evaluated together, we detected that our results are consistent with the results of both studies.<sup>5,7</sup> Serum GGT concentration was very high in colostrum in-

gested through suckling and it was reported to be a useful parameter to assess the passive transfer status of calves and lambs.<sup>15,17,18</sup> We aimed to determine whether GGT activity assessment is a useful method for determining FPT and whether any correlation with other parameters was observed in neonatal foals. The result of our study was found to be parallel with a study by Braun et al<sup>19</sup> who reported that GGT was not a useful method for determining the FPT in foals and we detected that there was no correlation between GGT and other parameters.

In conclusion, the results of this study clearly demonstrate that measurements of sIgG, STP concentration and the GCT duration before and after suckling are useful parameters for detecting FPT in foals. In addition, GCT and STP provide a simple and inexpensive field test. Simultaneously, the results of the present study showed that determination of GGT activity in foals is not a useful method to diagnose FPT for neonatal foals.

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