Bulgarian Journal of Agricultural Science, 19 (No 6) 2013, 1431-1436
Agricultural Academy

CONCENTRATION OF TOTAL IMMUNOGLOBULINS IN BLOOD AND MILK OF PERIPARTURIENT MARES AND THEIR TRANSFER TO FOALS VIA COLOSTRUM

S. BOBOS[1*], Lj. JOVANOVIC-PANIC[2], D. TRAILOVIC[3], M. PAJIC[1], Z. RASIC[4], M. RADINOVIC[1], A. GALFI[1] and S. SIMIN[1]

[1] *University of Novi Sad, Department of Veterinary Medicine, Faculty of Agriculture, 21000 Novi Sad, Republic of Serbia*
[2] *Higher Technical School, Department of Food Technology, 12000 Pozarevac, Republic of Serbia*
[3] *University of Belgrade, Faculty of Veterinary Medicine, 11000 Belgrade, Republic of Serbia*
[4] *Institute of Veterinary Medicine "Jagodina", 35000 Jagodina, Republic of Serbia*

Abstract

BOBOS, S., Lj. JOVANOVIC-PANIC, D. TRAILOVIC, M. PAJIC, Z. RASIC, M. RADINOVIC, A. GALFI and S. SIMIN, 2013. Concentration of total immunoglobulins in blood and milk of periparturient mares and their transfer to foals via colostrums. *Bulg. J. Agric. Sci.,* 19: 1431-1436

The aim of this study was to measure the importance of the humoral and cellular immune responses of mares' mammary glands, the impact of these responses on vitality and growth of newborn foals, to confirm the hypothesis that colostrum quality is the decisive factor for protection of newborn foals against neonatal infections and health in the first months of their life and that the time of and quantity of colostrum intake are more important factors in the optimal development of foal serum immunoglobulin (Ig) levels than the concentration of Ig in the colostrum. Seventeen mares and their newborn foals were investigated in this research. Mares were mated during 2008 and gave birth from February to May of the following year. All newborn foals were clinically examined and blood samples were collected from v. jugularis according to the following scheme: one hour postpartum, 24 hours postpartum and then once daily until the end of the first 7 days of life. Newborn foals were born with extremely low levels of gamma globulin (2.7 ± 1%). Satisfactory transfer of Ig was registered in 82% of the foals, while insufficient transfer of Ig was registered in 18% of the foals. The somatic cell count in milk of healthy mares was the highest on the first day postpartum and gradually declined over the next 7 days. In spite that, no correlation was observed between the somatic cell count and the establishment of cellular immunity in newborn foals.

Key words: mare, colostrum, foals, immunoglobulins, A/G ratio, somatic cell count

Abbreviations: Ig – immunoglobulin; A/G – albumin/globulin; SC – somatic cell; IgG – immunoglobulin G

Introduction

Adequate intake and absorption of required amounts of mare's colostrum, as well as the concentration of immunoglobulin (Ig) and colostrum quality in general, are obviously the most important factors for survival and growth of newborn foals. Abundant data in the literature is available on this subject, but it is almost exclusively related to English pure-bred horses. Thanks to modern and efficient diagnostic tools for the detection of Ig transfer disorders and the presence of various substitute Ig preparations, foal losses worldwide due to deficit of colostrum antibodies in newborn foals have been significantly reduced. Unfortunately, Serbia has a lack of appropriate prevention strategies for foal losses, especially in cases where the foals are unable to receive appropriate amounts of Ig rich colostrum. The key problem is the late and probably inadequate transfer of colostral antibodies to the foals.

E-mail: bobos@polj.uns.ac.rs

The aim of this study was to measure the importance of the humoral and cellular immune responses of mares' mammary glands, the impact of these responses on vitality and growth of newborn foals. For that purpose, two main tasks were:

- Detection of total protein concentration and individual protein fractions in colostrum and blood serum of newborn foals after colostrum intake and evaluation of the significance of factors affecting the colostral antibody transfer.
- Assessment of the correlation between immunoglobulin concentration in colostrum of mares and blood sera of their newborn foals.

Materials and Methods

Seventeen mares and their newborn foals were included in this investigation. Mares were mated during 2008 and gave birth from February to May the following year.

Blood samples were collected from the mares at two time points - 30 days and 7-15 days before the presumed parturition day.

All newborn foals were clinically examined and blood samples were collected from v. jugularis according to following scheme: one hour postpartum, 24 hours postpartum and then once daily until day 7. Simultaneously, all mares were milked to obtain colostrum for analysis.

All blood samples were centrifuged and sera were collected and frozen until biochemical examination of total protein and various protein fractions. Total protein concentration was determined using the colorimetric method, albumin levels were measured using the BCG spectroscopic method and electrophoresis was used for detection of serum protein fractions. After that, the albumin/globulin (A/G) ratio was calculated.

Colostrum samples were milked from both halves of the udder in approximate 30 mL volumes and analyzed for total protein concentration and various protein fractions, milk fat and somatic cell count. Protein concentration was determined using standard laboratory methods with colostrum protein fractions separated by agarose-gel electrophoresis.

Smears were made from each colostrum or milk sample, air dried and stained by the Newmann-Lampert method modified according to Levowitz-Weber for somatic cells count.

All results were statistically analyzed; mean values and standard deviation were calculated and compared with t-test to determine statistically significant differences. Calculated P values less than 0.05 were considered as significant. Statistical analysis was performed using the software package "Statistica version 10".

Results

The content of Ig in the blood serum of mares just before parturition (Table 1) is in accordance with the importance of secretion of colostral Ig.

Thirty days before the parturition and on parturition day, the albumin/globulin ratios were, on average, 1.7 ± 0.8 and 1.2 ± 0.5, respectively. The globulin concentrations thirty days before parturition, 7-15 days before parturition and on parturition day were 23.6 g/L, 27.7 g/L and 33.2 g/L, respectively.

In Table 2, the percentage ratio of different protein fractions in mare colostrum is shown.

Table 1
Total protein, albumin and globulin concentrations (x ± SD) in sera of mares before and on parturition day

Protein measured in sera of mares	30 days before parturition	7-15 days before parturition	Day of parturition
Total proteins, g/L	60.480 ± 20.420	58.250 ± 6.390	70.130 ± 12.560
Albumins, g/L	36.780 ± 6.362	30.718 ± 7.597	36.906 ± 6.137
Globulins, g/L	23.650 ± 6.314	27.755 ± 6.249	33.222 ± 1.268
A/G ratio	1.735 ± 0.777	1.191 ± 0.485	1.279 ± 0.602

A/G ratio - albumin/globulin ratio

Table 2
Percentage ratio of different protein fractions, separated by electrophoresis, in colostrum of mares (x ± SD) during the first seven days of lactation

Protein measured in mares colostrum, %	Lactation days			
	1	2	4	7
Prealbumins	11.555 ± 4.333	9.341 ± 7.190		
Albumins	2.666 ± 4.222	25.777 ± 3.900	34.421 ± 3.772	33.801 ± 4.981
Alpha globulins	4.555 ± 0.765	12.211 ± 1.003	26.443 ± 5.311	24.546 ± 5.433
Beta globulins	12.333 ± 4.356	14.999 ± 2.400	14.232 ± 2.228	12.333 ± 1.989
Gama globulins	53.431 ± 3.466	40.222 ± 4.767	18.667 ± 4.333	16.800 ± 4.566

Transfer of maternal Ig to the offspring certainly depends on the quality of colostrum (the concentration of Ig in the colostrums) and the amount of ingested colostrum (Tables 2 and 3), which is difficult to determine when the foal is nursing. For these reasons, attention in the current study then turned to the content of colostrums. The results in Table 3 clearly show that the dry matter content (primarily the protein fraction) in the first samples of the colostrum were significantly higher than in the samples taken 24 hours later, which is entirely consistent with the observation that the largest increases of Ig concentration in blood sera of newborn foals were observed in that particular period. Tables 3 and 4 summarize the colostrum composition of mares and the protein content in blood serum of foals. No direct relationship could be seen between the concentration of protein in the milk and the sera of foals.

Low levels of Ig were detected in all blood samples from the newborn foals regardless of whether the blood sampling was taken before the first nursing and regardless of the foals' intake of colostral antibodies. The average content of gamma globulin in foals' sera was 2.7 ± 1.9 % (Table 4).

It is significant that after 24 hours, the gamma globulin concentration had surpassed 4 g/L in eleven of the seventeen foals.

Table 5 shows the concentration of total protein, albumin and globulin in the blood sera of newborn foals during the first seven days of life. The albumin/globulin ratio proved to be a very valuable indicator of protein and immune status in newborn foals. Newborn foals had lower globulin than albumin levels prior to colostrum intake (Table 5), so the A/G ratio was greater than 6 (6.6 ± 5.2). After intake of colostral antibodies, the A/G ratio increased significantly, followed by a decrease to 3.0 (3.0 ± 1.6) 24 hours after birth, and further decreasing to normal values for adult horses (1.7 ± 0.6) after 7 days of life.

Besides being the source of antibodies, colostrum certainly plays a major role in the development of cellular immunity. Therefore, we measured the average number of somatic cells in colostrum and milk on the 1st, 2nd, 5th and 7th day of lactation as shown in Tables 6a and 6b.

Observation of the average somatic cell count in the milks from a homogeneous group of mares (mares with the excep-

Table 3
Protein, fat and water content in colostrum of mares (x ± SD) during the first seven days of lactation

Colostrum properties	Lactation days			
	1	2	4	7
Proteins, g/L	96.678 ± 19.456	63.456 ± 23.666	57.879 ± 17.567	49.422 ± 24.989
Fat, %	3.233 ± 0.333	2.187 ± 0.296	2.089 ± 0.167	2.188 ± 0.267
Water, %	83.233 ± 9.333	92.187 ± 8.296	92.089 ± 8.167	93.188 ± 7.267

Table 4
Individual protein fractions in the blood serum of newborn foals during the first seven days of life

Protein % in foals' serum	Days after parturition				
	1	2	3	5	7
Albumins	57.494 ± 5.472	46.000 ± 4.813	47.449 ± 6.257	44.972 ± 6.257	42.690 ± 2.737
Alpha 1-globulins	5.871 ± 2.671	9.731 ± 5.459	5.879 ± 3.024	3.750 ± 1.568	8.700 ± 5.454
Alpha 2-globulins	14.834 ± 6.404	15.466 ± 8.359	11.203 ± 3.762	12.900 ± 5.936	16.385 ± 2.946
Beta globulins	15.552 ± 4.711	13.416 ± 6.004	17.449 ± 5.558	17.152 ± 5.028	14.250 ± 7.017
Gama globulins	2.707 ± 1.896	14.322 ± 10.194	17.090 ± 14.827	18.268 ± 8.855	18.450 ± 2.860

Table 5
Total protein concentration, albumin and globulin concentration (x ± 1 SD) in blood serum of newborn foals during the first seven days of life

Proteins in foals' serum	Days after parturition				
	1	2	3	5	7
Total proteins, g/L	44.989 ± 4.684	47.019 ± 6.176	52.143 ± 7.682	50.575 ± 1.231	48.700 ± 7.237
Albumins, g/L	36.026 ± 4.366	33.483 ± 4.457	34.543 ± 8.986	31.433 ± 10.153	30.125 ± 5.889
Globulins, g/L	9.367 ± 5.207	13.644 ± 5.747	17.600 ± 7.762	18.633 ± 13.950	18.575 ± 4.936
A/G ratio	6.649 ± 5.177	3.028 ± 1.613	2.634 ± 1.903	2.007 ± 1.238	1.740 ± 0.646

A/G ratio - albumin/globulin ratio

Table 6a
Somatic cell count in colostrum and milk of mares during the first seven days of lactation

	Days after parturition			
	1	2	5	7
X	492 917.23	331 263.04	335 979.72	82 715.73
SD	988 949.85	636 091.79	633 591.39	151 284.69
CV	200.63	192.02	188.58	182.89
IV	48 861.0 - 2 510 882.2	29 997.0 - 1 626 504.0	31 130.2 - 1 468 719.7	12 342.11 - 353 298.0

X – arithmetic mean, SD - standard deviation, CV - coefficient of variation, IV - interval of variation

Table 6b
Somatic cell count in colostrum and milk of mares during the first seven days of lactation without data relating to the mare "Drina"

	Days after parturition			
	1	2	5	7
X	89 324.24	72 214.84	52 794.72***	15 070.17***
SD	29 537.42	49 711.35	25 016.01	3,150.09
CV	33.07	68.84	47.39	20.9
IV	48 861.00 - 126 654.00	29 997.00 - 157 762.00	31 130.20 - 74 459.20	1 342.11 - 17 798.22

X – arithmetic mean, SD - standard deviation, CV - coefficient of variation, IV - interval of variation, ***extremely significant

tion of the mare "Drina", the colostrum of which contained abnormally high levels of somatic cells, and so was drastically different from the average), clearly showed that the average somatic cell (SC) counts obtained progressively reduced from those counted on day 1 (89324.23 SC/mL or 89 x 10^3 SC/mL), day 2 (72 x 10^3 SC/mL) and day 5 (53 x 10^3 SC/mL) post-partum, reaching the lowest values on day 7 post-partum (15 x 10^3 SC/mL). Marked udder edema was observed in the mare "Drina" one week before parturition. In spite of this, the foal of this mare was thriving, with an optimal concentration of Ig, and white blood cell counts quite close to the average values.

There was no correlation between the somatic cell counts in the mares' milk (Table 6) and blood serum Ig levels of correlated foals (Table 5).

Discussion

Since foals are born without Ig due to the specific structure of the placenta, colostrum is a particularly important source, which provides antibodies necessary for survival of the newborns from parturition until acquisition of their own active immunity. Therefore, it is essential that the foal receive enough colostrum immediately after birth, since the ability for absorption of Ig decreases from hour to hour, to almost marginal levels 24 hours after birth, with a simultaneous reduction of Ig concentration in the colostrum.

Similarly, Dankow et al. (2006) and Axon and Palmer (2008) reported declines of the total protein concentration in the colostrum of Wielkopolska mares up to 1.74 times during the second day after parturition. Importantly, we have recorded a considerably higher average concentration of proteins and Ig in the first colostrum samples of the mares in this study than many other authors (we detected 96.7 ± 19.5 g/L on the first day compared to 63.5 ± 23.7 g/L on the second day). However, the latter figure corresponds with the findings of Ciesla et al. (2009), which confirms a suspicion that the first colostrum as well as blood samples, were, in many other studies, taken too early before the first suckling or immediately after the first suckling. Pasquini et al. (2005) also found high levels of total protein in the milk of mares at parturition (93.6 g/L), and a significant drop (to 60.2 g/L) 12 hours post-partum, which corresponds quite accurately to our observed value. Wlodarczyk-Szydlowska et al. (2005) and Csapo-Kiss et al. (1995) found even higher values of total milk protein (112.4 g/L and 164.1 g/L respectively), while Tishner et al. (1996) found lower values of total milk protein 12 and 24 hours after birth (36.4 g/L and 31.7 g/L, respectively).

In the current study, there was no correlation between the protein concentration in colostrum and blood serum of newborn foals (Tables 3 and 4), which only emphasizes the conclusion that the time and quantity of colostrum intake are more important factors than the concentration of Ig in the co-

lostrum. Of course, this applies to normal colostrum, since, clearly, colostrum by definition should not have critically low immunoglobulin concentrations. However, Trailović et al. (2000; 2003) indicated that foals which ingested less colostrum in the first hours of life, even though their mothers' colostrum was rich in immunoglobulin, had significantly lower concentrations of Ig than other investigated foals.

Production of Ig and their presence in the fetal serum of horses may be, according to Becht and Semrad (1985), detected at approximately 185 days of pregnancy. Because of fetal production, the detectable level of Ig in the blood serum of newborn foals is about 16 mg/dL before suckling (Giguere and Polkes, 2005). The presence of low IgG concentrations probably reflects the degree of intrauterine antigenic stimulation. A gamma globulin content of 2.7% can certainly be considered insufficient to protect the newborns, but this value must be interpreted with caution due to the influence of a few relatively high individual values, indicated by the high standard deviation in this study (Table 4).

Big individual discrepancies were also present in the following days, although, clearly, there was an increase in the concentration of gamma globulin 24 hours after the intake of colostrum (the average level, measured after 24 hours, was 14.3 ± 10.2 %), which probably indicates large variations between the time of first nursing and amount of the first colostrum intake (Table 4). Although, stabilization of Ig levels was detected on the fifth day, it is obvious that the transfer of colostral Ig is not strictly related to the first 24 hours, and there is slight increase of gamma globulin concentration on the third and fourth day of foal life (Table 4). However, the results of Giguere and Polkes (2005) indicate that even minimal quantities of Ig in the first day of life (4 g/L) are indispensable for the survival of foals.

The seventh day is considered as a threshold for the stabilization of all biochemical indicators, including the content of total protein and various fractions of serum proteins. We observed that the optimal concentration of total Ig of 8 g/L, set by Giguere and Polkes (2005), was attained in our foals on the fifth day, as Ig levels in blood sera were, on average, 9.3 g/L or 18.27 % (Table 4).

However, when we consider individual animals, three (18%) of the foals had gamma globulin concentrations below the minimum protective level on the seventh day, while six (36 %) of foals had very low concentrations of serum gamma globulin on the second day of life. Those three foals had insufficient transfer of colostral Ig related to their late and more difficult acceptance of nursing, and consequently lower colostrum intake, which suggests that amount of consumed colostrum and the time of first consumption are more important than the quality per se (Boboš and Vidić, 2005).

In most cases, the foal starts to nurse in the first 2 hours after birth, so the maximum transfer of colostral antibodies is expected in the first 18 hours of life (Jeffcott, 1974). The absorption of macromolecules is a non-selective process and it is done by pinocytosis, mostly in a short time after birth, but pinocytosis efficiency rapidly decreases to 22% of optimum after three hours and to less than 1% of optimum within 20 hours (Giguere and Polkes, 2005). This rapid decrease is caused by substitution of specialized enterocytes for Ig absorption with mature ones. However, it seems that late nursing delays maturation of enterocytes to some extent, which means that, in practice, a less vital foal could in fact use some of the Ig consumed later via colostrum.

The progressive reductions in somatic cell counts for the mares' colostrum we observed correlate with the results Danków et al. (2006), Harryman et al. (2013) and Wells et al. (2012).

High somatic cell counts values indicate the presence of a secretion disorder, and could be an indicator of mastitis. All mares in the current study, with the exception of the mare "Drina", which had udder edema, were clinically healthy and there were no signs of mastitis.

Conclusions

Analysis of the results obtained allowed the following conclusions to be drawn:

- The immune status of the mare plays a significant role in ensuring the quality of the colostrum. A significant increase in serum concentrations of total protein and globulin occurs one day before and at parturition day.
- Newborn foals are born with extremely low levels of gamma globulin (2.7 ± 1.9%). Transfer of Ig via colostrum is the most important prerequisite for the provision of necessary antibodies and a normal serum protein content.
- Insufficient transfer of Ig, registered in three (18 %) of the foals, was related to their late and more difficult acceptance of nursing and consequently, lower colostrum intake, indicating that the amount of consumed colostrum and the time of first consumption are more important than its quality.
- Transfer of colostral Ig depends on the quality of colostrum, the time of the first intake and the amount of consumed colostrum. Satisfactory transfer of Ig was registered in 14 of the 17 foals.
- The somatic cell count in the milk of healthy mares was the highest on day 1 postpartum and gradually declined over the next 7 days. In spite that, no correlation was observed between the somatic cell count and the establishment of cellular immunity in newborn foals.

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Received May, 15, 2013; accepted for printing September, 2, 2013.