

## IMMUNOTRANSFER IN THE FOAL

## LA TRASMISSIONE DELL'IMMUNITÀ NEL PULEDRO

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## SUMMARY

The study has been carried out on 10 crossbred mares belonging to the same breeding with the purpose of evaluating their seric and colostral immunoglobulins content and the relationships with the passive transfer received by their foals. Serum samples were collected at regular periods during the last month of pregnancy until delivery to assess the immunoglobulins and total proteins levels. IgG's serum concentration was about 2500 mg/dl at 30 days before delivery, about 2300 mg/dl one week before and 2020 at delivery, with a significative trend to decrease near foaling. Total proteins mean level was 6.01 g/dl, with a good correlation with IgG concentration. Mares serum and mares colostrum IgG were not significantly correlated. Colostral IgG level at foaling was more than 8000 mg/dl, nearly 6500 mg/dl 3 hours after and about 1300 mg/dl at six hours (15% of concentration at delivery). IgG(T) were strictly correlated with IgG and highest values were observed at foaling (about 1700 mg/dl). IgM and IgA contents were also highest at the same period (respectively 86 mg/dl and 623 mg/dl). A significative correlation was observed between IgG and IgM. Serum IgG mean values of 9 foals were nearly 200 mg/dl in the samples collected within three hours from the first suckling until to reach more than 2100 mg/dl at 18 hours. IgG(T) were significantly correlated with IgG and showed highest values at 18 hours. IgM and IgA contents also showed highest values between 12 and 18 hours. Significative correlation was found between foal serum IgG contents at 6 and 18 hours and colostrum IgG levels at delivery. Foal serum  $\alpha$ 1-globulins level significantly increased between 3 and 48 hours from birth; the same was noted for  $\gamma$ -globulins between 3 and 24 hours. Albumin/Globulins ratio decreased, during the first 24 hours, from 2.5/1 at birth until about 1/1 at 18-24 hours.

Key words: horse breeding, immunoglobulins, colostrum, foal, FPT.

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## RIASSUNTO

La ricerca è stata condotta su 10 fattrici meticce appartenenti allo stesso allevamento al fine di valutarne i contenuti immunoglobulinici sierici e colostrali e le relative connessioni con il trasferimento dell'immunità passiva al puledro. A tale scopo sono stati raccolti ad intervalli regolari campioni ematici durante l'ultimo mese di gravidanza fino al parto. La concentrazione sierica delle IgG è diminuita significativamente da circa 2500 mg/dl, 30 giorni prima del parto, a 2300 mg/dl circa la settimana antecedente ed a 2020 mg/dl al parto. I valori relativi alle proteine totali sono risultati correlati significativamente con i livelli di IgG. I valori delle IgG del siero delle madri e quelle colostrali non sono risultati correlati. Il contenuto colostrale di IgG è stato pari ad oltre 8000 mg/dl nei campioni presuckle ed è sceso a circa 6500 mg/dl dopo 3 ore ed a 1300 mg/dl circa nelle campionature della sesta ora, con una riduzione di circa l'85%. Le IgG(T) hanno evidenziato una stretta correlazione con le IgG, mostrando, anch'esse, i valori più alti nelle campionature operate al momento del parto (1700 mg/dl circa). Anche per le IgM e le IgA, allo stesso tempo di prelievo, si sono evidenziate le aliquote maggiori (86 mg/dl e 623 mg/dl, rispettivamente). Una correlazione significativa è stata osservata anche tra IgG ed IgM. I livelli sierici di IgG in 9 puledri indagati sono aumentati da circa 200 mg/dl, nei campioni raccolti entro le prime 3 ore dalla prima poppata, ad oltre 2100 mg/dl in quelli raccolti alla diciottesima ora. Anche in questo caso vi è stata correlazione significativa tra IgG(T) ed IgG. I valori più alti, per tutte le sottoclassi, sono stati raggiunti tra la dodicesima e la diciottesima ora. Una importante e significativa correlazione è stata individuata tra i contenuti sierici di IgG dei puledri a 6 ore e 18 ore ed i relativi livelli riscontrati nei campioni colostrali raccolti al parto. La quantità di  $\alpha$ -1 globuline è aumentata significativamente nel siero dei puledri dalle 3 alle 48 ore dalla nascita ed altrettanto si è verificato per le  $\gamma$ -globuline nel periodo 3-24 ore. Il rapporto albumina/globuline è diminuito, durante il primo giorno di vita dei puledri, passando da valori iniziali di oltre 2,5/1 a circa 1/1 nelle campionature raccolte a 18 e 24 ore.

Parole chiave: allevamento equino, immunoglobuline, colostro, puledro, FPT.

## INTRODUCTION

Foals are virtually agammaglobulinemic at birth needing colostrum suckling to gain an adequate passive transfer for their early protection against infective factors. Mares colostrum is the only immunoglobulins source for the newborn foal, since the diffuse epitheliochorial placenta doesn't allow the antibodies transfer during pregnancy (Mc Guire & Crawford, 1973; Steven & Samual, 1975; Perryman e coll., 1980). Foals are able to absorb different classes and subclasses of colostrum immunoglobulins (Igs) through specialized cells lining the small intestine during the first 18-24 h after birth; after this time absorption is not possible and this could cause FPT (Failure of Passive Transfer) (Mc Guire & Crawford, 1973; Jeffcott, 1974;

Morris et al., 1985; Waelchli et al., 1990), that is the most common cause predisposing sepsis in foal from birth to 4 weeks of age. The aim of this work is to evaluate mare serum, colostrum and foal serum Igs concentration; if any correlation exists between this three classes and between Igs and if it's possible to forecast, as soon as possible, an adequate immunotransfer from mare to the foal in order to prevent FPT.

## MATERIALS AND METHODS

10 crossbred multiparous mares belonging to the same breeding and feeding the same diet (4-5 kg oats, ad libitum medium hay and a proteic supplement during the last 3 months of pregnancy), and 9 foals (1 foal died within 72 hours), were employed for this experiment during a full reproductive season. All mares foaled in box stalls; foals were clinically normal at birth and suckled colostrum without assistance within 2 hours of parturition. Mares were immunized with inactivated rhinopneumonitis virus at 5, 7 and 9 months of gestation, and yearly with tetanus toxoid and influenza virus vaccine. Foals were given no vaccines during the observation period. Mares blood samples (10 ml) were collected at regular periods during the last month of pregnancy until delivery and in the first 48 hours after foaling to assess main Igs classes and total proteins (TP) levels; exactly time of blood collecting was: -720 hours (-30 days), -360 hours (-15 days), -168 hours (-7 days), 0 hours (delivery), and 6, 12, 24, 48 hours after foaling. Colostrum samples (20 ml) were collected at delivery (presuckle samples), at 3, 6, 12, 24, 48, 72 and 360 hours after foaling. Foals blood samples were collected within 3 hours of first suckling and after at 6, 12, 18, 24, 48, 72, 120 (5 days), 168 (7 d), 720 (30 d), 1080 (45 d) hours. All serum and colostrum samples were frozen within 2 hours and stored at -20°C until analysis were performed. Colostrum samples were centrifuged (4000 rpm at 5°C for 15 m) to remove cellular debris and fat and sometimes diluted before analyzing (1:4 in presuckle samples and 1:2 in all others) so that the diameters of the precipitin rings fell within the range defined by different Igs standards (1600 mg/dl for IgG). Serum TP were determined by the Biuret method (Henry, 1974). Colostrum TP were determined accord-

ing to FIL-IDF 20B: 1993 method. Serum and colostrum IgG, IgG(T), IgM and IgA concentrations were determined by use of single radial immunodiffusion (S.R.I.D.) (VMRD Inc, Pullmann, WA). Main serum protein fractions, albumin,  $\alpha 1$ ,  $\alpha 2$ ,  $\beta$  and  $\gamma$ -globulins were performed with Microtech 648R (INTERLAB, RM). Data were expressed as mean value  $\pm$  SD. MANOVA with repeated measures to evaluate significative differences at different collection times was performed by JMP (SAS Inst., 1994).

In order to examine the relationships between mares and foals and colostrum samples data were statistically investigated about Pearson linear correlation.

## RESULTS AND DISCUSSION

### *Mare serum*

Mare IgG's concentration (Tab. I) was 2461 mg/dl at 30 days before delivery, 2295 mg/dl one week before and 2020 mg/dl at delivery, with a significative trend to decrease nearly foaling; this fact is probably connected with the contemporary concentration of the same immunoglobulins fraction in mare colostrum, as reported by Jeffcott (1974), even if his observed IgG level was lower. Lavoie et al. (1989) found IgG amounts, at delivery, not different from ours (2233 mg/dl). At 12 h after foaling IgG level in our samples was 2050 mg/dl, and about 1900 mg/dl between 24 and 48 hours post-partum. De Meo Scotoni et al. (1992) didn't find significative differences between serum IgG levels before and after foaling in thoroughbred and local mares; Kohn et al. (1989), in the period of 12 hours after foaling, found a medium IgG level of 2463 mg/dl. We than note not unanimous opinions about the IgG level decrease at foaling, but we observed, by the consulted Literature, a significative blood IgG increment some weeks after foaling.

IgG(T) level was 506 mg/dl as mean value, with highest amount at delivery (593 mg/dl) and a good correlation with IgG ( $r = 0.73$ ,  $p < 0.01$ ); no significative differences were observed in the examined period. These amounts are similar to them found by Mc Guire and Crawford (1973) in blood samples collected at delivery and 1, 3, 16 days after.

**Tab. I.** Mean IgG, IgG(T), IgM, IgA (mg/dl) and TP (g/dl) mare serum levels at different samples collecting times (h).

Collection time h	IgG		IgG(T)		IgM		IgA		TP	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	sd
-720	2461	a 592	562	162	135	32	294	106	6.37	0.49
-360	2263	b 570	484	137	165	33	278	91	6.00	0.53
-168	2295	b 539	522	116	161	47	298	133	5.98	0.35
0	2020	bc 390	593	161	144	41	370	154	6.37	0.68
6	2033	bc 692	423	228	127	59	233	58	5.67	0.64
12	2043	bc 734	439	239	142	68	322	171	5.77	0.93
24	1900	c 630	530	202	116	33	296	135	5.92	0.91
48	1934	c 699	488	214	173	102	301	128	5.97	1.08

**Tab. II.** Mean IgG, IgG(T), IgM, IgA (mg/dl) and TP (g/dl) mare colostrum levels at different samples collecting times (h).

Collection time h	IgG		IgG(T)		IgM		IgA		TP	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
0	8867	a 1970	1758	a 701	86	41	623	296	17.11	a 4.11
3	6573	b 1888	1130	b 341	60	38	333	156	13.80	b 2.40
6	1283	c 366	316	c 120	37	28	210	89	6.30	c 0.92
12	659	d 693	128	d 152	30	28	184	71	4.15	d 1.23
24	205	e 113	54	e 21	17	18	141	27	3.40	e 0.62
48	176	60	69	23	11	4	135	26	3.21	0.48
72	152	62	52	24	7	5	124	22	3.03	0.30
360	114	24	52	2	2	1	122	21	2.41	0.16

IgM mean level was 146 mg/dl, with not significant differences between the samples collected during total experimental period. IgM/IgG mean ratio was around 7%, quite in the middle between results obtained by Mc Guire & Crawford (1973) (~8%) and by Kohn et al. (1989) (~5%).

IgA mean content was 299 mg/dl with a maximum of 370 mg/dl near foaling. Similar amounts are reported by Mc Guire & Crawford (1973), Kohn et al. (1989) and Pastoret et al. (1990).

Total serum proteins mean level was 6,01 g/dl and we observed a good correlation ( $r = 0.70$ ,  $p < 0.01$ ) between IgG (28% of TP) and TP. Cauvin et al. (1994) found IgG/TP ratio as 22.8%.

### *Colostrum*

IgG level in colostrum samples at foaling (Tab. II) was 8867 mg/dl, 6573 mg/dl 3 h after, 1283 mg/dl 6 h after (about 15% of concentration at delivery) 659 mg/dl 12 h after, and 205 mg/dl at 24 hours after foaling (about 2.3% of initial concentration) with significant differences. The decrease is not so dramatic until 15 days (114 mg/dl). The same results were obtained by Rouse & Ingram (1970) and Cash (1995). Meier et al. (1985) found higher amounts at 12 hours. In most cases Jones & Brook (1997a, 1997b) confirm that minimum IgG pre-suckle colostrum content would be 3800 mg/dl, even if sometimes foals fed a good kind of colostrum show serum IgG concentrations lower than 400 mg/dl at 24 h and were, consequently, considered FPT. Some other Authors (Higuchi et al., 1989; Koterba, 1990) confirm the poor quality of colostrum with a IgG concentration lower than 4000 mg/dl and Others (Pearson et al., 1984; Le Blanc et al., 1992) have observed differences within horse breeds.

IgG(T) and IgG levels are significantly correlated ( $r = 0.90$ ,  $p < 0.01$ ) in mare serum; highest values were observed at foaling (1758 mg/dl), lower three hours after (1130 mg/dl); these IgG(T) colostrum levels respectively correspond to 20% and 17% of the same period amounts IgG. Multivariate analysis by repeated measures shows significant differences between samples at delivery, and 3, 6, 12, 24 hours after.

IgM contents were highest at foaling (86 mg/dl), and a little lower (60 mg/dl) 3 h after, until reaching the lowest amounts (2 mg/dl) 15 days from delivery. Mc Guire & Crawford (1973); Kohn et al. (1989) and Lavoie et al. (1989) found 150 mg/dl, 123 mg/dl, 104 mg/dl,

respectively, in presuckle colostrum samples. Significant correlation ( $r = 0.69$ ,  $p < 0.01$ ) was found between IgM and IgG levels.

IgA colostrum contents were also highest at foaling (623 mg/dl), were 50% reduced (333 mg/dl), 3 hours after and 66% (210 mg/dl) at 6 hours. Correlation between IgA and IgG class was significant ( $r = 0.74$ ,  $p < 0.01$ ); both show a lowering trend, but at 15 days colostrum IgA amount is higher (122 mg/dl) than IgG one (114 mg/dl). In the horse, with the increasing milk production, the protective role of IgA on the oral, rhinopharyngeal and gastroenteric mucosa is underlined (Galan et al., 1986). IgA classes are really predominant in other equine secretions, like saliva and tears (Pahud et al., 1972; Vaermann et al., 1971).

Colostrum TP were also strictly correlated ( $r = 0.98$ ,  $p < 0.01$ ) with IgG, like in mare serum. The highest levels were found (17.11 g/dl) at delivery, with a significant falling down to 13.8 g/dl after 3 h and to 3.5 g/dl after 24 h, to reach typical milk values (2.41 g/dl) at 15 days. We have found, as reported by Waelchli et al., 1990, a decreasing ratio between IgG and TP according to the forwarding milk production, because of the quickness of IgG decrease. Significant differences were found between samples, particularly between foaling colostrum TP samples and 3 and 6 hours samples.

#### *Foal serum Igs and TP*

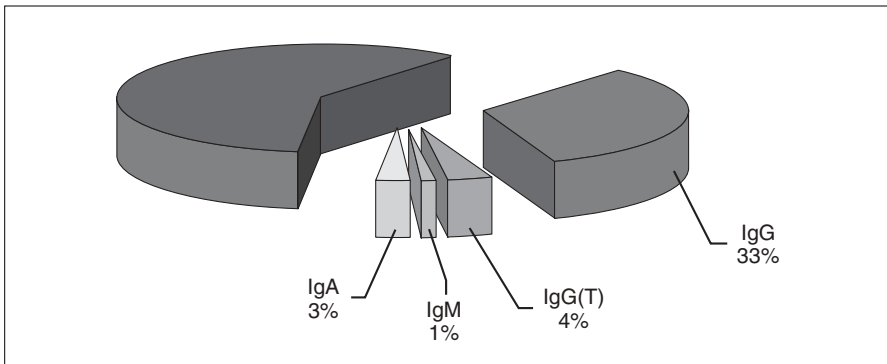
Foal serum IgG level (Tab. III) was 211 mg/dl in samples collected within 3 h from the first suckling, 539 mg/dl after 6 h, that is considered by the consulted Authors (Kähn, 1991; Bublitz et al., 1991; Claubough et al., 1991; Stoneham et al., 1991; Massey et al., 1992; Lee et al., 1992; Le Blanc et al., 1992; Kumaran et al., 1994) a good amount to avoid FPT, even if Takai et al. (1989) and Jones and Brook (1997a) found low Ig levels in serum foals without FPT problems and vice versa. Highest values, with significant differences, were obtained by samples collected at 18 hours (2106 mg/dl), when foal IgG reach the same levels as in adult representing 33% of TP (Graph. 1). From this period, we can observe a decrease at 24 h (1798 mg/dl), at 48 h (~1700 mg/dl) and 72 h (1446 mg/dl) until reaching 1172 mg/dl at 7 days from birth. Lee et al. (1992) have considered not significant 3 hours serum IgG level to forecast a possible FPT and more indicative 6 hours level.

**Tab. III.** Mean IgG, IgG(T), IgM, IgA (mg/dl) and TP (g/dl) foal serum levels at different samples collecting times (h).

h	IgG		IgG(T)		IgM		IgA		TP	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
3	211	a 108	67	9	22	9	54	41	3.76	a 0.22
6	539	b 118	96	29	28	7	91	32	4.35	b 0.41
12	1950	c 277	191	64	63	47	172	93	5.66	c 0.28
18	2106	d 279	204	82	68	50	152	51	5.76	c 0.33
24	1798	e 207	168	61	49	19	134	45	5.50	cd 0.50
48	1716	f 315	151	59	40	12	103	36	5.32	d 0.30
72	1446	265	151	64	40	14	98	36	5.09	d 0.33
120	1286	243	138	69	34	9	89	27	4.93	0.42
168	1172	354	139	69	32	9	76	24	4.78	0.49
720	853	327	122	42	41	12	75	18	4.87	0.48
1080	648	96	109	42	32	12	78	23	4.47	0.32

a, b, c, d, e, f: P &lt; 0.05





**Graphic 1.** Igs/TP ratio on foal serum samples at 18 h collection time.

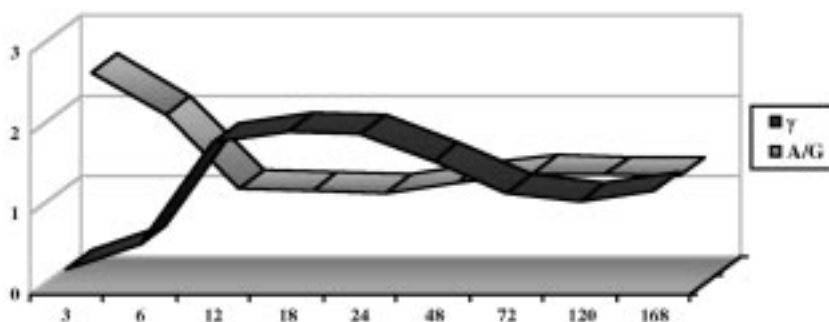
IgG(T) were significantly correlated with IgG ( $r = 0.60$ ,  $p < 0.01$ ), as found by Mc Guire & Crawford (1973), and showed highest values (204 mg/dl) at 18 hours. IgG(T)/IgG ratio was 32%, 3 hours after first suckling, and decreases at 9.8% at 12 and 18 h.

IgM and IgA contents also showed highest values between 12 and 18 h (63 mg/dl, 68 mg/dl for IgM; 172 mg/dl and 152 mg/dl for IgA, respectively). IgM/IgG ratio was 3.2% at 12-18 hours, with the decrease (2.3%) at 48 h. IgA/IgG ratio was 8.8% at 12 h and also decreases at 48 h (6%). A good correlation was observed between IgM and IgG levels ( $r = 0.55$ ,  $p < 0.01$ ) and between IgA and IgG amounts ( $r = 0.52$ ,  $p < 0.01$ ).

TP level was 3.76 g/dl, 3 h from the first suckling, according to the results obtained by Rumbough et al. (1978), to reach highest amounts (5.76 g/dl) at 18 hours, with significative difference. A good correlation is underlined between serum TP and IgG ( $r = 0.85$ ,  $p < 0.01$ ), so that some Authors (Rumbough et al., 1978; Tyler Mc Gowan et al., 1997) suggest the possibility to forecast FPT by evaluating TP serum content, because the close correlation between TP and IgG serum content, moreover when IgG level reach highest values. On this way, we can observe, by our investigations, that IgG represent 5.6% of TP in the 3 h samples and 36% in the 18 h ones.

#### *Foal serum protein fractions*

Albumin amount is included between 2.62 g/dl and 2.77 g/dl and no significant differences were observed.  $\alpha$ 1-globulins amounts sig-



**Graphic 2.**  $\gamma$ -globulins (g/dl) and Albumin/Globulins ratio at different collection times (h).

nificantly increased between 3 hours and 48 hours from delivery. Trumel et al. (1996) indicate similar  $\alpha_2$  and lower  $\alpha_1$  amounts. Not different results are indicated from the same AA for  $\beta$ -globulins contents.  $\gamma$ -globulins significantly increased between 3 hours and 18-24 hours from delivery. A/G ratio was 1.06 and 1.02, respectively at 18 and 24 hours, decreasing from 2.53 at 3 hours, due to progressive  $\gamma$ -globulins concentration in the foal (Graph. 2).

### *Correlations*

No significant correlations were found between whole mare serum Ig classes and foal serum immunoglobulins.

Mare colostrum IgG at delivery and mare serum IgG at delivery ( $r = 0.74$ ,  $p < 0.05$ ) and at 48 h ( $r = 0.95$ ,  $p < 0.05$ ) were significantly correlated.

Mare colostrum IgG at foaling and foal serum IgG at 6 h ( $r = 0.84$ ,  $p < 0.05$ ) and 18 h ( $r = 0.91$ ,  $p < 0.05$ ) were significantly correlated.

Mare colostrum IgG 3 h after delivery and foal serum IgG content at 6 h ( $r = 0.90$ ,  $p < 0.01$ ), at 12 h ( $r = 0.96$ ,  $p < 0.01$ ), at 18 h ( $r = 0.88$ ,  $p < 0.01$ ), at 24 h ( $r = 0.93$ ,  $p < 0.01$ ) were also significantly correlated. Similar correlations were found by Morris et al. (1985) and Da Luz et al. (1992) and represent the most important result in our work. By the examination of mare presuckle colostrum IgG levels, or collected at most in the first three hours, on the basis of our preliminary investigations, we could forecast the normal IgG foal serum level and, when lower amounts are discovered, feed the foal extra-colostrum in the first 20 hours or treat with equine plasma transfusion.

## CONCLUSIONS

Mare serum IgG levels were always higher than 2000 mg/dl before delivery and became lower at delivery, probably because of the progressive concentration of the same antibodies in mare colostrum. We have observed a good correlation between total IgG and TP in mare serum. IgG colostral content showed highest level at delivery with a 85% reduction after 6 h and about 90% after 12 h. It's absolute necessary for the foal to suckle within this period. Lower IgG colostrum content than 3800-4000 mg/dl is considered poor and inadequate to prevent FPT. IgG and TP colostrum contents were correlated and their ratio decreased with increasing lactation. IgG(T) were strictly correlated with IgG in mare colostrum and in foal serum. IgA were also significantly correlated with IgG in colostrum and became predominant during the proceeding lactation. Foal serum IgG content increased until reaching the highest values at 18 h. It's very important to verify an higher IgG level than 400-500 mg/dl at 6 h from the first suckling. We have observed significative correlations between foal serum IgG and IgG(T), IgM, IgA. IgM/IgG ratio was 3% and IgA/IgG ratio 7-8% between 12 and 18 h. Nevertheless is not possible to define all functions of differences Ig classes, even if current Literature (Perryman et al., 1987; Weldon et al, 1992) indicates a selective foal IgM immunodeficiency. We have also observed a good correlation between foal serum IgG and TP, but the most important result of this work showed a significative correlation between mare colostrum IgG content at delivery and foal serum IgG content at 6h and 18 h and mare colostrum IgG 3 h after delivery and foal serum IgG content at 6, 12 and 18 h. The early forecast of possible FPT could get the foal able to be fed with extra-colostrum, or, later, treated with equine plasma transfusion. We have found an average of 5.6 g/dl total proteins and 2.8 g/dl total globulins with a medium albumin/globulins ratio as 1. The foal died within 72 hours showed a low IgG serum levels (less than 200 mg/dl), TP as 3.6 g/dl, 1.42 g/dl globulin level, with a A/G ratio as 2.5 and normal mare serum and colostral levels. The evolution of foal serum proteins was also very important, particularly related to  $\gamma$ -globulins concentrations increasing and A/G ratio decreasing and we consider the evaluation of these parameters as a complement of Igs determination, to have a complete opinion of adequate immunity transfer from mare to the foal.

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