İstanbul Üniversitesi
Veteriner Fakültesi Dergisi

Journal of the Faculty of
Veterinary Medicine Istanbul University

İstanbul Üniv. Vet. Fak. Derg. / J. Fac. Vet. Med. Istanbul Univ., 41 (2), 129-137, 2015
doi: 10.16988/iuvfd.2015.94892

Araştırma Makalesi ***Research Article***

Clinical Trials during Late Pregnancy in Mares: Prevention of Abortion

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Geliş Tarihi / Received:
22 April 2014

Kabul Tarihi / Accepted:
17 November 2014

Anahtar Kelimeler:
Vitamin-amino asit bileşenleri, *Bifidobacterium bifidum*, yavru atma, önleyici metodlar, kısraklar

Key Words:
Vitamin-amino acid supplement, *Bifidobacterium bifidum,* abortion, methods of prevention, mares

Abstract

The paper observed the influence of the integrated treatment of abortion in mares with the supplement Haemobalans® to replace high-turnover vitamins, amino acids and minerals; and probiotics BF-15® which contains *Bifidobacterium bifidum*. The aims of this study were to prove the application of Haemobalans and BF-15 as adaptogens during late gestation. We examined 60 healthy adult Thoroughbred mares that were divided into four equal groups (n=15) on the 270 - 300th days of gestation. Test animals received the following treatment: 1 intramuscular injection of Haemobalans in 3 days, in a dose of 1 ml per 45 kg of body weight. BF-15 was also given for 10 days: 20 ml of the oral solution with oats two times per day. Reflex-1 group was treated with the same dose of Haemobalans. Reflex-2 group was treated with the same dose of BF-15. Control animals were also examined. Blood samples were taken before the treatment and two weeks after it. First examination showed the basic level of investigated sources, the last one showed the result of the treatment. The difference between the data (which were obtained before and after treatment) was compared in each group. In case of significant differences between the data of the test group relative to the other three groups, we talked about the reliability of the outcomes. Tissue of placentae were taken after delivery, fixed with neutral formalin and stained with hematoxylin and eosin. Zones of pathological changes were identified. Database of categorical data was created in placental study. Number of cases for markers of placental insufficiency (PI) was determined. In the personal database of one animal the presence of a pathological case was marked (1) and the absence was marked (0). The objective excess of (1) was regarded as PI. The concentrations of glucose, total protein, creatinine, hemoglobin, vitamin B12, ESR, count of lymphocyte and MCHC in the test group were significantly higher: 16.4%; 10.78%; 17.87%; 11.7%; 9.5%; 14.7%; 22.2%, 10.5% higher respectively ($P<0.05$; in relation on the other groups). In the histological study, placentae of mares which were treated with Haemobalans held proliferation and vascular congestion of villi. Histological changes of placentae in the control group and the reflex-2 group included hyperemia and diapedesis into chorionic villi, protein degeneration of syncytiotrophoblast, shortened villi. Thus, the complex of medications improves metabolism better than the use of a single medicine.

Özet

Kısraklarda Son Gebelik Dönemindeki Klinik Çalışmalar: Yavru Atmaların Önlenmesi

Çalışmada kısraklardaki düşüklerin yüksek-dönüşüm hızlı vitaminlerin, amino asitlerin ve minerallerin yerine geçmesi amacıyla bir tamamlayıcı olan Haemobalans® ve *Bifidobacterium bifidum* içeren probiotik BF-15 ile entegre tedavisinin etkisi araştırılmıştır. Çalışmada, geç gebelik döneminde uygulanan Haemobalans ve BF-15 gibi adoptojenlerin etkilerinin ispat edilmesi amaçlandı. Çalışmada, gebeliğin 270 - 300. günlerinde dört eşit gruba (n=15) ayrılan 60 sağlıklı erişkin İngiliz kısrak araştırıldı. Kullanılan kısraklara 3 gün içinde, her 45 kg vücut ağırlığına 1 ml dozda olacak şekilde Haemobalans® intramüsküler yolla uygulandı. BF-15 de 10 gün süreyle günde 2 kez yulaflı 20 ml oral solüsyon olarak verildi. Reflex-1 grubu aynı doz Haemobalans ile tedavi edildi. Reflex-2 grubu aynı doz BF-15 ile tedavi edildi. Kontrol hayvanları da incelemeye alındı. Tedaviden önce ve tedaviden iki hafta sonra kan örnekleri alındı. İlk inceleme araştırılan kan değerlerinin temel düzeylerini gösterdi, son inceleme ise tedavinin sonucunu gösterdi. Her grupta veriler arasındaki fark (tedaviden önce ve sonra elde edilen) karşılaştırıldı. Test grubundaki verilerin diğer üç gruba oranla anlamlı farklı olduğu durumda, sonuçların güvenirliği tartışıldı. Doğumdan sonra plasenta dokusu alındı, nötral formalin ile sabitlendi ve hematoksilin ve

eosin ile boyandı. Patolojik değişim alanları belirlendi. Plasenta çalışmasında kategoriksel bilgilerin veritabanı oluşturuldu. Plasental yetersizlik (PI) belirteçleri için vaka sayıları belirlendi. Bir hayvanın kişisel veritabanında patolojik vaka mevcudiyeti (1), eksikliği ise (0) olarak belirtildi. (1)'in objektif olarak fazlası PI olarak kabul edildi. Test grubunda glukoz, total protein, kreatinin, hemoglobin, B12 vitamini, ESR, lenfosit sayısı ve MCHC anlamlı olarak daha yüksek bulundu: tespit edilen değerler sırasıyla %16,4, %10,78, %17,8, %11,7, %9,5, %14,7, %22,2, %10,5 (P<0,05). Histolojik çalışmada, Haemobalans ile tedavi edilen kısrak plasentalarında proliferasyon ve villuslarda vasküler konjesyon oluştuğu gözlendi. Kontrol grubunda ve Reflex-2 grubundaki plasentaların histolojik değişiklikleri arasında koryonik villuslara diapedesis ve hiperemi ile villuslarda kısalma ve sinsitiyotrofoblastlarda protein dejenerasyonu, tespit edildi. Bu sonuçlar ışığında, çoklu ilaç kullanımının metabolizmayı tek ilaç kullanmaktan daha iyi onardığı kanısına varıldı.

Introduction

The purpose of a successful reproductive program is to produce the maximum possible number of healthy foals each year from the pool of mares, as was noted by Knottenbelt (2003). Pregnancy loss limits equine reproductive ability. The rate of late pregnancy loss is about 17%, as was noted by Hong et al. (1993). Stressing conditions such as pour ration, infrequent feeding or overtraining influence the pregnancy outcome and can be a cause of abortion.

Importantly, the domestication of the horse has led to fundamental changes in environmental conditions. However, the nature of horses has not changed from the ecological point of view. It has been reported by Plemyashov (2010) that treatment which is aimed at reducing the harmful effects of the industrial environment is the successful strategy in cases of pregnancy disorders.

It is important to know, while providing therapeutic and preventive measures, that the system of mother – fetus consists of two separate organisms united under a common goal - successful development of the fetus, as was noted by Fedorova and Kalashnikov (1986). These organisms affect each other, varying the parameters of metabolism, including metabolism of biologically active substances. Ultimately, these changes lead to a different perception of the treatment modalities, as was noted by Filippov (2009).

Normal development of the fetus is determined by the constant coordination of the functions of two organisms: the mother and the fetus. The main link between them is the placenta. This complex of tissues cannot be independent because of the absence of its own mechanisms of autonomic regulation. However, the mother - fetus system becomes mature and functionally active only in the presence of a complete placenta, as was noted by Giles (1993).

Available theoretical and practical data show that the placenta is able to selectively accumulate, synthesize, and pass the necessary substances to the body of the fetus. It is involved in nutrient-metabolic and endocrine processes, as was noted by Karpenko (2006). It is well studied that biologically active substances can integrate in this cycle of metabolism, as was noted by Karpenko and Andreev (2007).

The role of biologically active substances during pregnancy is so important that the change in their amounts can control the whole pregnancy, as was reported by Parey (2005).

The metabolism of biochemically active substances in drugs during pregnancy goes in a more complicated way than usual. The concentration of the drugs or their metabolites in the body of the fetus is affected by several factors: 1) pharmacokinetics and pharmaco-dynamics of drugs in the body of the mother; 2) transfer of the drug across the placenta and its metabolism into the placenta; 3) distribution, metabolism and excretion of the drug from the body of the fetus; 4) reabsorption of amniotic fluid, as was reported by Filippov (2009), Samper et al. (2006) and Smolenskaya-Suvorova (2009). All these factors were taken into account when creating Haemobalans.

The possibility to get necessary nutritional resources from feeding determines the successful outcome of pregnancy. It can be controlled by providing probiotics, as was noted by Potapova et al. (2014). This group of medicine helps to support the metabolism of biochemically active substances and improve local immune system of intestinal tract. It is very important to avoid complications in the digestion to prevent cases of colic in mothers.

The use of probiotics has a positive effect on the metabolism, improving membrane digestion and increasing the digestibility of forages. It also prevents gynecological disorders as it is a microflora donor for gastro-intestinal and urogenital tract; normalization of microflora of the environment, as in the faeces significantly reduced allocation of opportunistic and pathogenic organisms. Consequently, at birth the foal will receive mainly normal microflora from the birth canal and from the environment, as was noted by Danilevskaya (2004), Trafalska and Grzybowska (2004).

Thus, there is an amount of clinical evidence that confirms the relevance of the task for the development science-based technology to improve the safety of foals through the correction of the clinical status of the maternal organism, as was reported by Glade (1991), Shefer (2010) and Whitwell (2001).

According to these scientific data, the clinical trial was based on application of adaptogenic substances which support the adequate nutrition in a mare for

normal fetal development during late gestation. The treatment consisted of intramuscular injection of Haemobalans, which contains a mixture of vitamins, amino acids and minerals, and application of oral solution BF-15, which contained a culture of *Bifidobacterium bifidum* and their metabolites as sources of amino acids.

The purpose of this clinical study was to prove the integrated application of Haemobalans and BF-15 as adaptogens during late gestation and to verify the result with the biochemical analysis of serum and the histological analysis of the fetal part of placentae. The main criterion of a successful clinical trial was safekeeping of neonatal foals of the tested mares. The rates of abortion or stillbirth were also determined.

Materials and Methods

The design of the study and the terms of use of the pharmacon in animals were chosen in accordance with GLP rules and international recommendations of ICH M3. The work with animals was carried out in accordance with Geneva Convention “Guiding Principles for Biomedical Research Involving Animals”. All manipulations with animals were performed by qualified veterinary doctors. Thus, animal experimentation was approved by the respective local authority.

Experimental Animals and Equipment

The clinical outcomes of the use of Haemobalans and BF-15 were studied at St. Petersburg State Academy of Veterinary Medicine. The study was performed on 60 adult healthy Thoroughbred mares on the 270 – 300th days of gestation. The animals were born and kept in the same breeding stable. The stable was protected from infectious diseases. Sporadic cases of staphylococcal arthritis and bacterial gastroenteritis were observed in newborn foals.

Good breeding histories of the tested animals were studied in their veterinary documentation. The fact of current pregnancy was proven with the rectal method.

The diet of the pregnant mares consisted of grain oats (6-7 kg/day), hay (10 kg/day), carrots, (3 kg/day), plenty of green grass in the summer, salt was used as a mineral supplement.

The dosage form of Haemobalans (Nature Vet, Australia) is a sterile solution for injection. Haemobalans comprises: L-lysine hydrochloride - 20 mg/ml; DL-methionine - 20 mg/ml; Glycine - 20 mg/ml; Ferric ammonium citrate - 15mg/ml; Cobalt sulfate - 240 pg/ml; Copper sulfate - 70 µg/ml; Riboflavin (vitamin B2) - 10mg/ml; Choline bitartrate (Vitamin B4) - 10 mg/ml; Pyridoxine hydrochloride (vitamin B6) - 10 mg/ml; Inositol (vitamin B8) - 10 mg/ml; Cyanocobalamin (vitamin B12) - 150 mcg/ml; Nicotinamide - 100 mg/ml; D-Panthenol - 15 mg/ml; Biotin - 10 µg/ml.

The dosage form of BF-15 (Neva Vet, Russia) is an oral solution with *Bifidobacterium bifidum* in the amount of not less than 15 ppm per 1 ml as well as organic acids, micro- and macroelements and vitamins, which are involved in metabolic processes of *B. bifidum*.

Experimental Design

We examined 60 healthy adult Thoroughbred mares that were divided into four equal groups (n = 15) on the 270 – 300th days of gestation. Test animals received the following treatment: 1 intramuscular injection of Haemobalans in 3 days, in a dose of 1 ml per 45 kg of body weight. BF-15 was also given for 10 days: 20 ml of the oral solution with oats two times per day. Reflex-1 group was treated with Haemobalans in the same dose. Reflex-2 group was treated with BF-15 in the same dose. Control animals were also examined.

Verification

The assessment of the results is based on the morphological and biochemical analysis of blood samples and the quality of the placental structure.

Blood samples were taken before the first injection of Haemobalans and two weeks after the last one. It was taken from jugular vein. The zone of injection was treated with ethanol 70%. We used vacuum tubes Vacuette® (Austria). There were two technical types of tubes for morphological and biochemical study. Blood samples were kept at 23 °C for 10 min and placed in a special refrigerator Thermos® (Canada) for transportation to the laboratory (temperature was 5 °C for not more than 12 hours).

The concentrations of total protein, glucose, blood urea, cholesterol, bilirubin, hemoglobin and serum iron levels were estimated with colorimetric method with the use of the commercial test-system "NPF Contour +". The concentrations of creatinine in serum and cyanocobalamin were photocolorimetry estimated with the use of the commercial test-system SPC "EcoService". Morphological analysis of blood was carried out with the use of the automatic system "Vision Hema".

Tissue samples of the placenta fetal part were obtained after normal parturition. Pieces of tissue were cut through the entire thickness of the placenta. Either normal tissues of placentae or a normal section with an adjacent abnormal locus, if any, were captured. Placental tissues were fixed with neutral formalin, dehydrated in alcohol and embedded in paraffin through chloroform. The thickness of serial paraffin sections was 5-7 microns. Pieces of placenta (gravid and nongravid horns) were stained with routine hematoxylin and eosin

in accordance with Merkulov (1961), Sapozhnikov and Dorosevich (2000). The criteria used for diagnosing a placenta were dystrophy, vasculature and quality of trophoblast cells. The specimens were examined with the light-optical microscope "Carl Zeiss", magnification at 100, 200. Microphotography was performed with the digital camera "Axio Scope A1".

Statistical Analysis

Blood samples were taken before the treatment and two weeks after it. First examination showed the baseline level of the investigated substances, the last one showed the result of the treatment. The difference between the data (which were obtained before and after the treatment) was compared in each group. In case of significant differences between the data of the test group relative to the other three groups, we talked about the reliability of the outcomes. Tissues of placentae were taken after delivery. Zones of pathological changes were identified. A database of categorical data was created in placental study for each animal. The number of cases for markers of placental insufficiency (PI) was determined. In the personal database of one animal the presence of a pathological case was marked (1) and the absence was marked (0). The objective excess of (1) was regarded as PI. Two cuts for each animal were analyzed. Not less than 40 visual fields were evaluated for each animal.

The data were statistically processed by "Statistica 7". Mean value (M) and standard deviation (SD) were determined. The appropriate differences among the means were compared by paired Student's T-test. The differences were considered significant at $P<0.05$ and $P<0.01$. The statistic methods were based on the recommendations of Middleton (2013).

Results

All the animals in each treatment groups remained in good health throughout the study. There were no signs of inappetence, diarrhea or other toxic symptoms.

Hematological studies, which were carried out before the treatment, showed no statistically significant differences between the groups (Table 1). This indicated the statistical homogeneity of variances. The data were considered as the baseline level of the blood parameters.

As regards the hematological and biochemical parameters, statistically significant changes were showed in different groups. These data are presented in Table 2.

Table 1. Parameters of blood samples before treatment.
Tablo 1. Tedavi öncesindeki kan değerleri.

Parameters	Groups			
	Test (M ± SD)	Reflex-1 (M ± SD)	Reflex-2 (M ± SD)	Control (M ± SD)
Glucose, mmol/l	4.20 ± 0.31	4.17 ± 0.33	4.13 ± 0.32	4.16± 0.33
Total protein, g/l	68.58 ± 2.6	62.97 ± 2.8	67.06 ± 2.4	69.15 ± 2.3
Creatinine, mmol/l	121.28 ± 8.6	116.54 ± 8.1	119.34 ± 7.3	120.12 ± 6.32
Blood urea, mmol/l	5.30 ± 0.5	5.44 ± 0.6	5.41 ± 0.6	5.24 ± 0.7
Total bilirubin, mkmol/l	27.8 ± 2.9	29.4 ± 2.3	31.1 ±3.8	30.6 ± 2.3
Bilirubin conjugated, mkmol/l	7.08 ± 0.7	7.13 ± 0.8	6.37 ± 0.5	6.59 ± 0.4
Serum iron, mkmol/l	27.48 ± 3.4	27.39 ±2.9	24.6 ± 2.4	25.2 ± 1.8
Cholesterol mlmol/l	2.53 ± 0.26	2.38 ± 0.13	2.37 ± 0.64	2.2 ± 0.11
Vitamin B12, pg/ml	3262.66 ± 85	3681.31 ± 91	3417.84 ± 93	3312.8 ± 89
Erythrocytes, 10^{12}/l	8.47 ± 0.34	8.52 ± 0.76	8.61 ± 1.09	8.83 ± 0.44
Hemoglobin, g/l	100.25 ± 6.8	100.37 ± 6.4	98.37 ± 5.9	98.9 ± 6.8
ESR, mm/h	65.75 ± 6.8	65.45 ± 6.5	64.1 ± 6.3	63.00 ± 6.4
Hematocrit, l/l	0.39 ± 0.02	0.39 ± 0.03	0.40 ± 0.02	0.41 ± 0.05
Leukocytes, 10^{9}/l	8.1 ± 0.6	8.0 ± 0.6	8.0 ± 0.9	7.9 ± 0.7
Lymphocytes, 10^{9}/l	25.25 ± 0.9	24.13 ± 0.9	25.91 ± 0.8	23.14 ± 1.0
MCHC, g/l	242.54 ± 3.5	258.84 ±4.7	249.39 ± 3.4	270.25 ± 3.5

Table 2. Parameters of blood samples after treatment.

Tablo 2. Tedavi sonrasındaki kan değerleri.

Parameters	Groups			
	Test (M ± SD)	Reflex-1 (M ± SD)	Reflex-2 (M ± SD)	Control (M ± SD)
Glucose, mmol/l	5.99 ± 0.4**	5.91 ± 1.4**	5.27 ± 0.83**	5.01 ± 0.5
Total protein, g/l	77.13 ± 15.2**	74.40 ±12.1**	73.57 ± 12.6**	68.81 ± 12.4
Creatinine, mmol/l	146.3± 25.2*	157.11 ± 29.5*	139.74 ±17.6**	120.14 ±21.2
Blood urea, mmol/l	6.76 ± 0.8***	5.99 ± 1.2***	6.14 ± 0.6***	5.92 ± 0.5
Total bilirubin, mkmol/l	16.44 ± 3.3*	17.13 ± 9.9*	22.75 ± 4.25**	26.75 ± 3.2
Bilirubin conjugated, mkmol/l	5.51 ± 0.26*	4.99 ± 0.5*	5.31 ± 0.4**	6.17 ± 0.5
Serum iron, mkmol/l	25.85 ± 8.7***	26.31 ± 4.9***	25.97 ± 4.7***	25.77 ± 5.7
Cholesterol, mlmol/l	2.41 ± 0.2***	2.43 ± 0.8***	2.48 ± 0.7***	2.44 ± 0.2
Vitamin B12, pg/ml	3942.39 ± 107**	3882.67 ± 90**	3692.47 ± 85**	3567.27 ± 93
Erythrocytes, 10^{12}/l	8.45 ± 0.5***	8.62 ± 0.5***	8.52 ± 0.33***	8.50 ± 0.6
Hemoglobin, g/l	110.8 ± 2.8**	106.8 ± 6.8**	99.9 ± 7.2**	97.80 ± 17.7
ESR, mm/h	61.30 ± 6.9**	62.45 ± 6.7**	57.80 ± 6.5**	52.25 ± 5.8
Hematocrit, l/l	0.39 ± 0.04***	0.39 ± 0.05***	0.40 ± 0.04***	0.38 ± 0.03
Leukocytes, 10^9/l	8.1 ± 1.2***	8.0 ± 1.3***	7.8 ± 0.9***	7.8 ± 0.8
Lymphocytes, 10^9/l	24.18 ± 1.6**	18.99 ± 1.7**	22.25 ± 0.8**	18.80 ± 2.1
MCHC, g/l	311.52 ± 13.2**	310.02 ± 13.5**	281.75 ± 13.5**	278.80 ± 12.7

*:P<0.01; **: P<0.05; ***: P>0.05 - differences between the data of the test group relative to the control group.

The concentrations of glucose, total protein, creatinine, hemoglobin, vitamin B12, ESR, count of lymphocyte and MCHC in the test group in the second samples were significantly higher: 16.4%; 10.78%; 17.8%; 11.7%; 9.5%; 14.7%; 22.2% and 10.5%, higher respectively (P<0.05) in relation to the first samples. Concentration of total bilirubin was 62.7% lower than in control group in; conjugated bilirubin concentration was 12.8% lower in the test group in the second samples in relation to the first samples.

No significant differences were obtained in the contents of blood urea, serum iron, cholesterol, erythrocytes, leukocytes and hematocrit (P>0.05).

The concentrations of glucose in the blood serum in the reflex-1 and the reflex-2 groups in the test group in the second series of samples were significantly higher: 15.23% and 4.90% than in the control group, respectively; total protein concentration was 7.51% and 6.40% higher; creatinine concentration was 23.5% and 14.02% higher in, total bilirubin concentration was 56.15% and 17.8% lower; direct bilirubin concentration was 23.64% and 16.19% lower; vitamin B12 concentration was 8.4% and 3.39% higher; hemoglobin concentration was 8.2% and 2.1% higher; ESR was 16.3% and 9.6% higher; count of lymphocyte was 15.5% and 17.1% higher; MCHC was 10.05% and 1.04% higher.

The greatest increase of glucose concentration was achieved in the test group (5.99 ± 0.4 mmol/l; P<0.05). On the other hand, the smallest increase was observed in the reflex-1 and the reflex-2 groups, which only used BF-15 (5.27 ± 0.83 mmol/l in reflex-2 group in comparison with 5.01 ± 0.5 mmol/l in the control group; P<0.05). The greatest increase of total protein concentration was achieved in the test group (77.13 ± 15.2 g/l; P<0.05). A significant increase was also observed in the reflex-1 and the reflex-2 groups (74.40 ± 12.1 g/l and 73.57 ± 12.6 g/l respectively in comparison with 68.81 ± 12.4 g/l in the control group; P<0.05). Increased concentrations of serum creatinine occurred in all treated groups. However, the greatest increase was observed in the reflex-1 and the reflex-2 groups where was used only Haemobalans (157.11 ± 29.5 mmol/l in comparison with 120.14 ± 21.2 mmol/l in the control group; P<0.05). The greatest increase in the concentration of vitamin B12 was in the test group (3942.39 ± 107 pg/ml in comparison with 3567.27 ± 93 pg/ml in the control group; P<0.05). However, the increase of concentration of vitamin B12 was observed in all the treated groups. The greatest improvement in red blood cells was achieved with the use of Haemobalans (MCHC – 311.52 ± 13.2 g/l in the test group; 281.75 ± 13.5 g/l in the reflex-1 group, 278.80 ± 12.7 g/l in the reflex-2 group and 310.02 ± 13.5 g/l in the control group; P<0.05). The greatest improvement in white blood cells was achieved with the use BF-15 (24.18 ± 1.6 10^9/l lymphocytes in the test group; 18.99 ± 1.7 10^9/l in the reflex-1 group; 22.25 ± 0.8 10^9/l in reflex-2 group; 18.80 ± 2.1 10^9/l in the control group; P<0.05).

None of the treated mares had significant histological changes of placentae structure. In the histological study, placentae of mares which were treated with Haemobalans held proliferation and vascular congestion of chorionic villi. It was associated with the angiogenic effect of the substance. Histological changes of placentae in the control group and the reflex-2 group included hyperemia and diapedesis into chorionic villi, protein degeneration of syncytiotrophoblast, shortened villi. Trophoblast layer was thinned; trophoblast cells did not assemble into functional knots and produced a small amount of syncytium. There were edematous connective tissues with collagen destructors and vasodilatation of internal vessels. The results of histological study are shown in figure 1 and 2.

The proof of adaptogenic effects of the integrated treatment is based on the determination of mortality rate during the antenatal period, which is shown in Table 3. The acquired data are not statistically sufficient; however, they demonstrate the tendency of the increase of the vital ability of foals in the antenatal period due to the integrated treatment

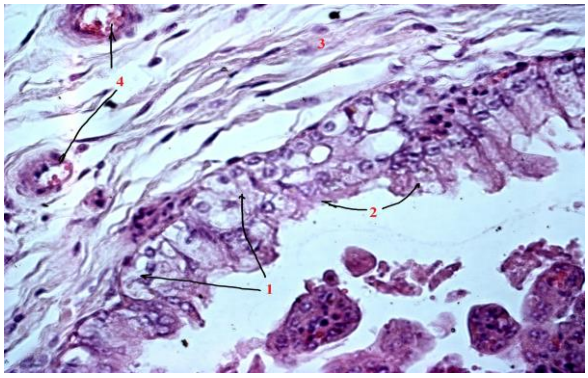

Figure 1. Abnormal chorionic surface. The section of placenta from a non-treated group. Stained with H and E, magnification is 200x. Trophoblast cells (1) are not packed in a knot, small, spread on the surface, they produce a little syncytium (2). The stroma (3) is edematous, dissociation of collagen fiber, contains expanded vessels (4).

Şekil 1. Anormal koryonik yüzey. Tedavi görmeyen gruba ait plasenta kesiti. H ve E ile boyalı, büyütme 200x. Trofoblast hücreleri (1) bir düğüm içinde paketlenmemiş, küçük, yüzey üstünde yayılmış, küçük bir sinsityum (2) oluşturmuş. Stroma (3) ödemli, kollajen lifte dağılma, genişletilmiş damarlar (4) içeriyor.

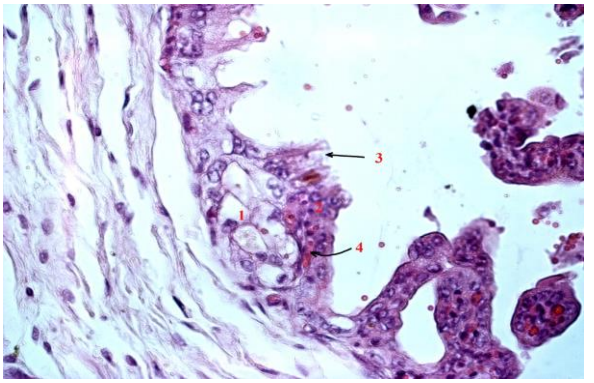

Figure 2. Normal chorionic surface. The section of placentae from a Haemobalans-treated group. Stained with H and E, magnification is 200x. Large trophoblast cells produce (1) syncytium (2) which is in good condition. Syncytium cells have ciliums (3). (4) - fetus`s blood vessels.

Şekil 2. Normal koryonik yüzey. Haemobalans ile tedavi edilmiş bir gruptan plasenta kesiti. H ve E ile boyanmış, büyütme 200x. Geniş trofoblast hücreleri (1) iyi bir durumda olan sinsityum (2). sinsityum hücrelerinin siliumları var. (3) (4) - fetusun kan damarları.

Discussion

Many researches pay their attention to metabolic disorders as the basic cause that could affect a pregnancy outcome and foal well-being. They confirm that the development of the fetus depends on the mother. In consequence, any increase or decrease of functional ability of the dam`s organism has a correlative effect on the fetus, as was noted by Karpenko (2006); Salamon et al. (2009). Methods of detection of metabolic disorders have high sensitivity and sufficient accuracy to be recommended for use in the diagnosis of reproductive ability of mares. Thus, we determined the effects of treatment with biochemically active substances using the biochemical analysis as the main method.

The results of this method were confirmed by the current literature. Most scientists say about the advisability of vitamins as treatment and prevention of pathologies in foals. We improved metabolism of vitamins of group B by using Haemobalans which is reflected in hematopoiesis, growing of fetus and maturity of placental tissues. It can be discussed in relation to the following references.

Table 3. The rate of abortion and stillbirth.

Tablo 3. Düşük ve ölü doğum oranı.

Parameters		Groups			
		Test	Reflex-1	Reflex-2	Control
Abortion	**N**	0	1	0	4
	%	0.0	1.66	0.0	6.66
Stillbirth	**N**	0	1	3	6
	%	0.0	1.66	5.0	10.0
Healthy newborn foal	**N**	15	13	12	5
	%	100.0	86.6	80.0	33.3

N – is numbers of cases; % - percent of cases from all outcomes.

- Cyanocobalamin is highly biologically active, as a growth factor which contributes to the development of normal hematopoiesis, maturation of red blood cells and the accumulation of the necessary compounds for the transport of oxygen. It also contributes to the regulation of carbohydrate and lipid metabolism, as was noted by Karpenko et al. (2008).
- Pyridoxine also has a definite place in metabolism. It is required for normal functioning of the central and peripheral nervous systems due to the synthesis of GABA, for the metabolism of essential amino acids and fats, as was noted by Filippov (2009)**.**
- Riboflavin plays an important role in energy metabolism as a coenzyme. Nicotinamide is a part of many enzymes. Panthenol plays an important role in the interstitial metabolism. Biotin is involved in various metabolic reactions responsible for skin and hair development, as was noted by Karpenko (2006).

According to many researchers, pregnant mares suffer from deficiency of vitamins. Subclinical vitamin deficiency does not lead to clinical manifestation of hypovitaminosis and is not accompanied by the development of specific well-defined symptoms, as was noted by Filippov (2009).

Speaking of protein metabolism, it is necessary to pay attention to the essential amino acid methionine which is contained in Haemobalans. It is the presence of the movable methyl group in the methionine molecule which causes its lipotropic effect. Methionine activates phospholipid synthesis and deposition in the neutral liver fats; they activate the synthesis of steroid hormones and affect the synthesis of a number of enzymes and vitamins, as was noted by Fedorova and Kalashnikov (1986), Filippov (2009).

Glucose is the main source of energy for fetus. We provided it by Haemobalans, too. The function of the brain tissue is largely dependent on the concentration of glucose in the blood serum. Glucose readily crosses the placenta, even in the presence of placental insufficiency. The transition of the mother's blood glucose into the blood of the fetus also increases oxygen consumption by fetal tissues, as was noted by Fedorova and Kalashnikov (1986), Filippov (2009) and Salamon et al. (2009).

Glucose has a stimulating effect on the utero-placental circulation, protecting fetus from hypoxia, as was noted by Fedorova and Kalashnikov (1986), Filippov (2009) and Karpenko (2006).

The lack of oxygen has a negative effect on fetal development as well as the lack of nutrients. A predisposing factor of the development of fetal hypoxia is abnormalities of blood circulation, reducing the concentration of hemoglobin and red blood cells in the blood serum.

We corrected the iron deficiency anemia by using Haemobalans, which allowed to increase the viability in antenatal period, as was noted by Fedorova and Kalashnicova (1986). The impact only on hematopoietic system is not a comprehensive solution in the case of fetal hypoxia. The improvement of the morphological status of placental complex tissues can increase the oxygen supply of the fetus, as was noted by Samper et al. (2006). Thus, we proved angiogenic effects of Haemobalans using the histological study of placentae.

In the study of histological tissue sections of the placenta treated with the drug Haemobalans showed proliferation and congestion of vessels of chorionic villi, which is associated with the angiogenic effect of the drug.

Adaptive-compensatory reactions found in the placenta tissue of mares in the control group are characterized by the expansion of blood filling of vessels and villi, diapedesis with cells, stromal edema, degenerative changes in the syncytiotrophoblast.

Numerous methods for diagnosis of pregnancy disorders provide the information of the polietilogic

nature of the problem, as was noted by Plemyashov (2010). It is difficult to identify the specific cause of development of a pregnancy disorder; thus, the problem requires the use of an integrated solution, as was noted by Fedorova and Kalashnikova (1986). Besides, the use of complex biochemically active medicines in the late pregnancy can mediate the increase of immunity in the fetus. This helps to limit the use of antibiotics in newborn foals.

It is not desirable to use antibiotics in newborn foals because of unpredictable deleterious effects. In addition, the uncontrolled use of antibiotics brings out a risk of appearances of resistant strains of microorganisms, as was noted by Danilevskaya (2004). The application of environmentally safe products, such as BF-15, is a good alternative to antibiotic therapy in neonatology. Clinical studies suggest that probiotics can be useful in stimulation of the immune system and control of gastrointestinal tract inflammatory diseases, as was noted by Trafalska and Grzybowska (2004). It was indirectly proven by reductions in infant mortality.

Histological analysis of placenta is the important diagnostic method because placental structure directly reflects the conditions in which the fetus developed. The research was to identify and describe the characteristic structure of placentae. The obtained results revealed a dependence between the structure of the fetal part of the placenta and reproductive function of mares. We obtained positive results of complex therapy with drugs Haemobalans and probiotic complex BF-15 for the correction of errors of metabolism in tissues of the placenta and blood samples, confirmed by the research of many authors on the effectiveness of this group of drugs in obstetric practice, such as Ousey and Rossdale (1993).

Sum it up, it is important to pay more attention to the composition of substances based on biochemical data. The integrated approach ought to take into consideration the internal pharmacological interaction of components. Haemobalans contains the important for fetoplacental well-being B vitamins and does not contain potential teratogenic vitamin A and E as was noted by Filippov (2009). The components of Haemobalans join into metabolic, processes in placenta preventing placental insufficiency. BF-15 normalizes the work of intestinal tract, decreases the risk of colic episodes and improves feed digestibility, as was researched by Potapova et al. (2014). Besides, tissues of placentae in the treated groups did not show areas of inflammation or cancer changes, which indicate the absence of teratogenic effects of the method.

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