Journal of Animal and Veterinary Advances 12 (3): 344-351, 2013
ISSN: 1680-5593

Microflora of the Mare Vagine in Norm and Abnormal

[1]Alimbekova Meruyert, [1]Julanova Nursulu, [1]Julanov Mardan,
[2]Kasenova Gulmira and [3]Myrzaliyev Akhan
[1]Department of Obstetrics, Surgery and Biotechnology of Animal Reproduction,
[2]Department of Biological Safety, Kazakh National Agrarian University, Almaty, Kazakhstan
[3]Department of Brucellosis, Kazakh Research Institute of Veterinary, Almaty, Kazakhstan

Abstract: The study is devoted to unresolved issues of veterinary gynecology. Changes of bacterial microflora of genitals is one of the reasons of infertility and is the problem reproduction in thoroughbred horse breeding. The solution of this problem requires a series of experiments to be carried out for definition of the taxonomical characteristic of vagina microorganisms of healthy and sick mares of English thoroughbred riding, Arab, achaltekin breeds of various age. Standard methods of bacteriological researches and electronic microscopy were applied. Mares were proved to be one of the reservoirs of infection secreting microbes in environment with genitals fluxes. In this respect, healthy animals as bacteria carriers pose threat of emergence of gynecological diseases. Microbiological landscape of vagina of mares and role of normal vaginal microflora are defined. It has been found that opportunistic pathogenic bacteria prevail with advancing age, level of lactic-acid bacteria is decreased which promotes development of pathological changes in vagina.

Key words: Bacteriological researches, vaginal lavage, reproductive function, postnatal period, microorganisms, gynecological pathologies

INTRODUCTION

Livestock farms are abundant in micropopulation including abnormal, modified under the influence of external factors (antibiotics, mating, etc.). According to Shin *et al.* (1979), Ricketts *et al.* (1993), Reid (1999), Kwon *et al.* (2012), specified microorganisms cause various negative changes in organism including genitals. According to Redaelli and Codazza (1977), Blue (1987), Le Blanc (1997) and Albihn *et al.* (2003) changes of bacterial microflora of genitals are attributed to the most likely and encountered symptoms of gynecological pathologies and pose a problem of reproduction in thoroughbred horse breeding.

Researchers saw the solution of this problem in definition of the taxonomical characteristics of vaginal microorganisms of healthy and sick mares which differs from research conducted by the above researchers. The results and findings of the research will form a basis for development of effective therapy and prevention of disorders of vaginal microflora of mares of thoroughbred breeds.

MATERIALS AND METHODS

Study was conducted on mares of the English thoroughbred riding, Arab, achaltekin breeds pertaining to horse breeding farms of Kazakhstan. Bacteriological research used vaginal lavage of healthy and gynecologic sick mares of different age groups (3-5, 6-8 and 9-12 years).

Vaginal lavage were received with observance of sterility by means of pig insemination device POS-5 (Russia). Before application a bottle of the device was filled with 50 cm^3 of physiological solution and sterilized together with a catheter in a water bath within 30 min at temperature 110°C. After that the catheter end was wound with sterile cotton wool and wrapped with parchment study from outside.

Before receiving the lavage from vagina of mares Furacilin solution 1:5000 was used for toilet of external genitals dried up with dry paper towel. After that interlabial space was opened and catheterized through the upper wall all the way in to vesical cervix. After that the bottle was raised at the level of sacral vertebras, squeezed out the contents in vagina. In a minute the bottle was turned over and lowered below the level of vulva and loosened. In this fashion injected physiological solution was drawn off back into the bottle together with vaginal contents. Further, the sample catheter was extracted with its end wiped with 70°C alcohol, wound with sterile cotton wool, wrapped with parchment study from outside and transported back to laboratory for research.

Standard techniques of microbiological researches were applied during execution of work. Four consecutive

Corresponding Author: Alimbekova Meruyert, Department of Obstetrics, Surgery and Biotechnology of Animal Reproduction, Kazakh National Agrarian University, Almaty, Kazakhstan

dilution procedures were made in test tubes (1:10, 1:100, 1:1000, 1:10000) with sterile physiological solution to receive germ culture of vaginal lavage.

Lavage samples were inoculated on meat-peptonnyyagar, MRS agar (DE Man, Rogosa, Sharpe), lactic acid bacilli cultivation media, nutrient medium from lactary hydrolyzate, Blourock medium for follow-up of bifid bacteria, Buchin medium for cultivation of corynebacteria, medium with sodium azide for fecal streptococci, Baird-Parker medium for staphylococci, wort agar, Sabouraud's medium for fungi, Endo medium, bismuth-sulfite agar for enterobacteria. The sporogenesis ability of microorganisms was studied either. Calculation of the grown colonies was carried out on the counter of moruloids. Swabs were prepared from per diem cultures, painted by Gram and investigated with the microscope.

Microoorganisms were also the object of study of cultural and morphological signs by inoculation for beef-extract broth, beef-extract agar and beef-extract gelatin. Biochemical activity of cultures was studied by inoculation of Hiss medium. Biochemical properties were determined by enterobacteria ability to carbohydrates fermentation. Identification of microorganisms and their taxonomical distribution was carried out in compliance with bacterium indicator by Bergey and Bergey's Manual of Systematic Bacteriology (Boone *et al*., 2005).

Petri dishes with inoculation were incubated in the thermostat at temperature 37°C in 48 h. Typical single moruloids were re-inoculated in the test tubes with beef-extract agar and differential and diagnostic nutrient media. Each colony was inoculated in three test tubes and placed in the thermostat at temperature 37°C. Pure growth was observed in 48 h.

Material preparation for electronic microscopy was carried out by classical technique. During laboratory investigation the following equipment was used: steam sterilizer VK-75-01 OKP 945121000206 (autoclave) manufactured in Russia, drying chamber ShS-80-01 SPU TU 9452-010-00141798-2005 manufactured in Russia, electric dry-air thermostat TSO-1/80 SPU TU 9452-004-00141798-2000 manufactured in Russia, water bath manufactured in Russia, transmission electron microscope JEM-1011 completed with CCD digital photocamera Morada (OLYMPUS) «JEOL», manufactured in Japan as well as digital binocular biological microscope of Motic BA 200 manufactured in Austria.

RESULTS

Researchers investigated 157 mares including 70 pregnant mares and 87 barren mares. Non-pathogenic and opportunistic pathogenic microorganisms were found in vaginal lavage of mares of all age groups. Findings of investigation are presented below in Table 1. Therefore, vaginal microflora of healthy most often contained lactic-acid bacteria (75.5-88.8%) and Sarcina (73.3-81.4%). Pathogenic microflora was not found in animal lavage.

Young mares (3-5 years) had non-pathogenic microorganisms of genus *Corynebacterium* (77.7%), *Bifidobacterium* (61.1%) of coccus family representatives of geni *Diplococcus* (31.4%), *Streptococcus* (25.9%) including α-streptococcus (11.1%), γ-streptococcus (5.5%) and γ-staphylococcus (16.6%) (Fig. 1-4).

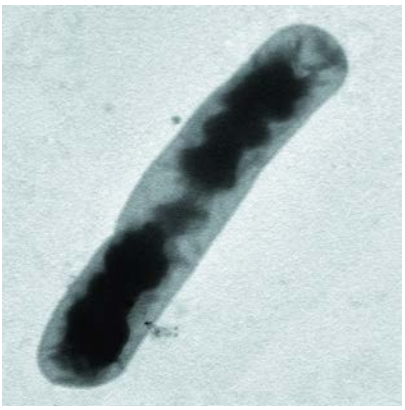

Fig. 1: Corynebacterium increase 12000 nm; transmission electron microscope JEM-1011

Table 1: Results of bacteriological research of vaginal lavage of healthy mares

	Age of mares (No. of animals)					
	3-5 years (54)		6-8 years (58)		9-12 years (45)	
Microorganisms	No. of animals	Percentage	No. of animals	Percentage	No. of animals	Percentage
Corynebacterium	42	77.7	44	75.8	29	64.4
Lactobacterium	48	88.8	51	87.9	34	75.5
Bifidobacterium	33	61.1	43	74.1	29	64.4
Sarcina	44	81.4	46	79.3	33	73.3
Diplococcus	17	31.4	15	25.8	9	20.0
Streptococcus	14	25.9	13	22.4	10	22.2
α-streptococcus	6	11.1	7	12.0	5	11.1
γ-streptococcus	3	5.5	7	12.0	5	11.1
γ-staphylococcus	9	16.6	10	17.2	5	11.1

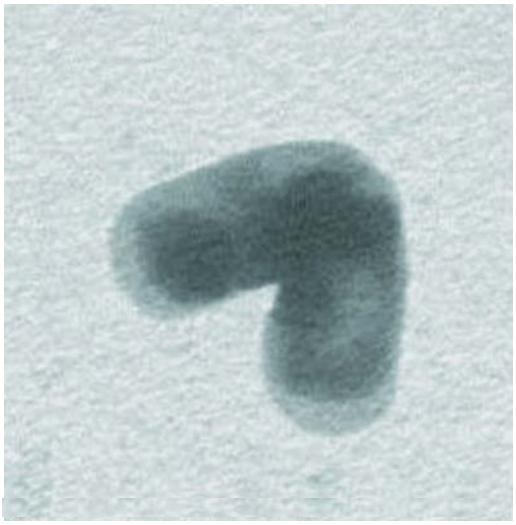

Fig. 2: Bifidobacterium increase 4000 nm; transmission electron microscope JEM-1011

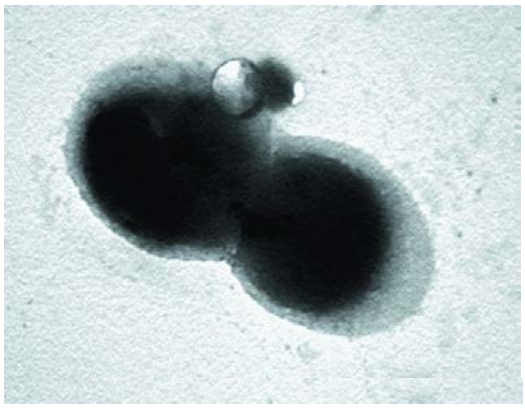

Fig. 3: Dipilococcus increase 15000 nm; transmission electron microscope JEM-1011

The investigation of vaginal lavage of 6-8 years old mares has shown microflora of geni *Corynebacterium* with ratio 75.8%, *Bifidobacterium* -y 74.1%, *Lactobacterium* -y 87.9% (Fig. 5) which in their turn were attributed to geni *Lactobacillus acidophilus*, *Lactobacterium casei*, *Lactobacterium plantarum* (Fig. 6-8) by morphological, cultural and some other physiological peculiarities. There was also a globulous bacterium of micrococcus group, Sarcina (79.3%) Fig. 9. From coccus family in this group were found Diplococcus (25.8%) and Streptococcus (22.4%).

Production of α-streptococcus in this group was slightly higher than in earlier (12%). Level of γ-streptococcus in this group was identical with

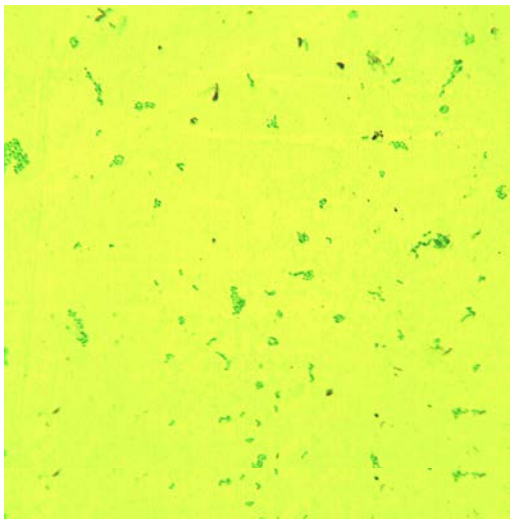

Fig. 4: Streptococcus increase x100; Digital binocular biological microscope of motic BA 200

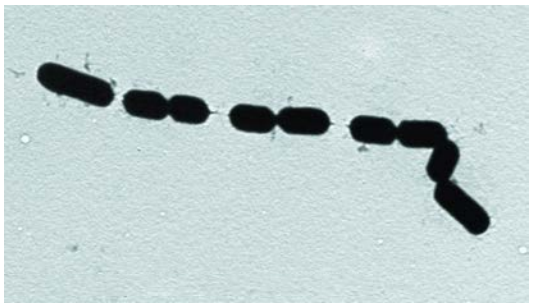

Fig. 5: Lactobacterium increase 8000 nm; transmission electron microscope JEM-1011

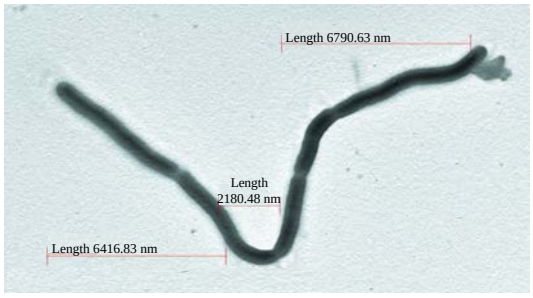

Fig. 6: *Lactobacillus acidophilus* increase 6000 nm; transmission electron microscope JEM-1011

γ-streptococcus nevertheless this value was 6.5% more than in the first age group. As for growth of γ-staphylococcus the difference with the first group made only 0.6%.

During investigation of vaginal lavage of 9-12 years old mares, it should be noted that the quantity of non-pathogenic microorganisms containing in normal vaginal microflora was lower than vaginal lavage of 3-5 and 6-8 years old mares. Therefore, Corynebacterium in vaginal lavage of mares of this age group was present in 6 4.4%, Lactobacterium in 75.5% and Bifidobacterium in 64.4% of mares. Sarcina was found in vaginal lavage of 73.3% of mares. From coccus families were found 20% Diplococcus, 22.2% Streptococcus, 11.1% α-streptococcus, 11.1% γ-streptococcus and 11.1% γ-staphylococcus.

The characteristic of vaginal microflora of mares with clinical symptoms of gynecological diseases is presented in Table 2.

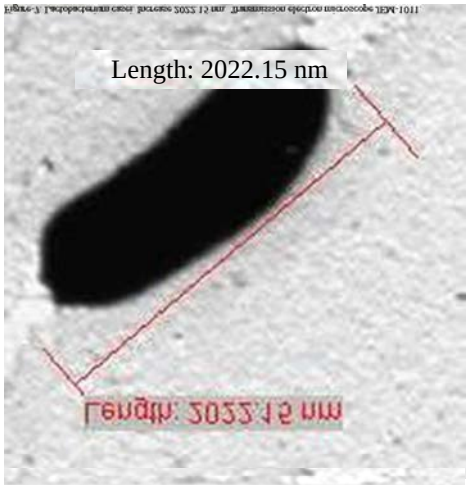

Fig. 7: *Lactobacterium casei* increase 2022.15 nm; transmission electron microscope JEM-1011

Researchers also found that opportunistic pathogenic microorganisms prevail in vaginal microflora of mares with clinical symptoms of gynecological diseases (Streptococcus, Staphylococcus, *Escherichia coli*, mold fungi, yeast and thread bacteria).

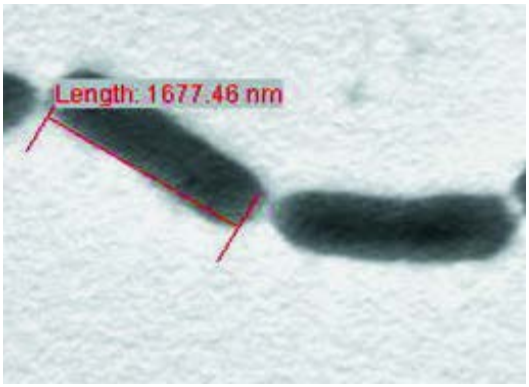

Fig. 8: *Lactobacterium plantarum* increase 1677.46 nm; transmission electron microscope JEM-1011

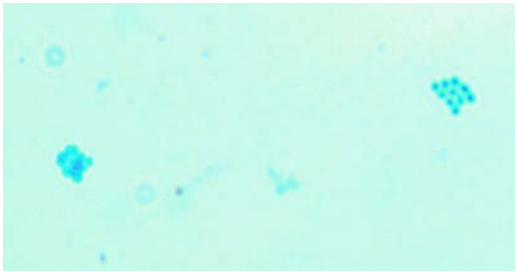

Fig. 9: Sarcina increase x100 digital binocular biological microscobe of motic BA 200

Table 2: Findings of bacteriological research of vaginal lavage of clinically sick mares

	Age of mares (No. of animals)					
	3-5 years (54)		6-8 years (58)		9-12 years (45)	
Microorganisms	No. of animals	Percentage	No. of animals	Percentage	No. of animals	Percentage
Corynebacterium	4	57	3	33	1	16
Lactobacterium	3	42	4	44	2	33
Bifidobacterium	1	14	3	33	-	-
Sarcina	5	71	4	44	3	50
Diplococcus	2	28	4	44	3	50
Streptococcus	2	28	5	55	3	50
α-streptococcus	-	-	2	22	1	16
γ-streptococcus	-	-	1	11	1	16
γ-staphylococcus	1	14	2	22	-	-
Staphylococcus	1	14	5	55	3	50
Escherichia	2	28	4	44	4	66
Sporogenous bacteria of the genus *Bacillus*	3	42	4	44	4	66
Yeast	5	71	7	77	5	83
Mold fungi	5	71	6	66	5	83
Thread bacteria	3	42	8	88	4	66

Samples of vaginal lavage of mares of age group 3-5 years showed that opportunistic pathogenic microorganisms included 42% sporogenous bacteria of genus *Bacillus*, pathogenic microorganisms included 71% yeast and mold fungi and 42% thread bacteria.

From the coccus family 28% of samples contained Diplococcus and Streptococcus and 14% Staphylococcus (Fig. 10). From 28% of samples were found *Escherichia coli* (Fig. 11 and 12).

In 57% of samples got from vagina of sick mares were reported non-pathogenic Corynebacterium, in 42% of samples were found Lactobacterium and 14% of samples contained Bifidobacterium. In 71% of samples was found globulous microflora from groups of micrococci, i.e., Sarcina.

During investigation of vaginal lavage of gynecologic sick mares of age group 6-8 years were found non-pathogenic microorganisms (Corynebacterium 33%, Lactobacterium 44% and Bifidobacterium 33%). In 44% of samples was found globulous microflora from groups of micrococci, i.e., Sarcina.

Also, 44% of samples contained Diplococcus, 55% Streptococcus where by 22% α-streptococcus as well as 11% γ-streptococcus. Staphylococcus was inoculated in 55% of samples. From 44% of samples contained *Escherichia coli*.

From opportunistic pathogenic microorganisms 4 of samples included sporogenous bacteria of genus *Bacillus* (Fig. 13) and from pathogenic microorganisms 77% accounted for yeast (Fig. 14), 66% for mold fungi and 88% for thread bacteria.

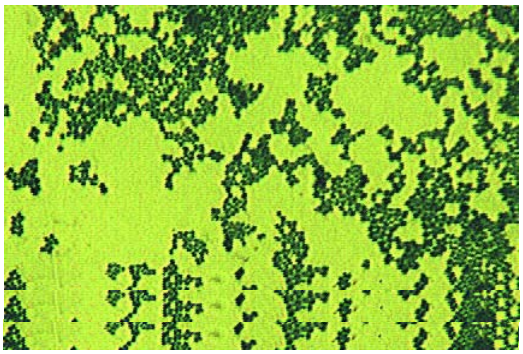

Fig. 10: Staphylococcus increase x100 digital binocular biological microscobe of motic BA 200

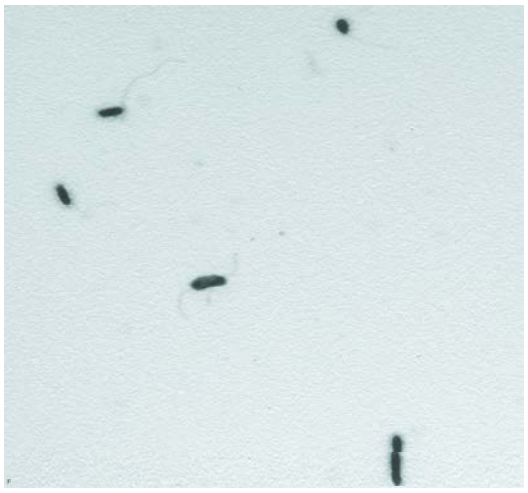

Fig. 11: *Escherichia coli* increase 3000 nm; transmission electron microscope JEM-1011

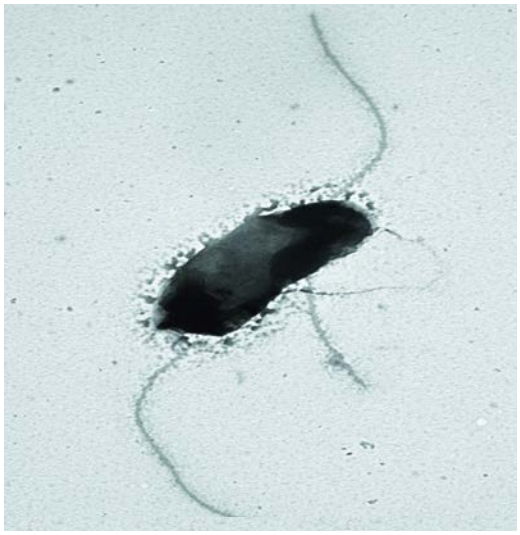

Fig. 12: *Escherichia coli* increase 20000 nm; transmission electron microscope JEM-1011

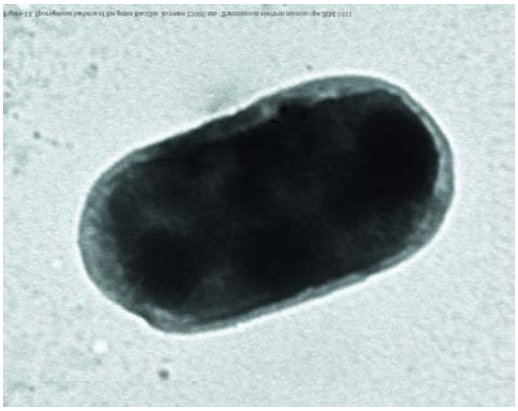

Fig. 13: Sporogenous bacteria of the genus *Bacillus* increase 25000 nm; transmission electron microscope JEM-1011

During investigation of vaginal lavage of clinically insane mares of age group 9-12 years 16% of samples included non-pathogenic microorganisms, namely Corynebacterium and 33% of samples contained Lactobactecrium. In distinction from indicators of samples collected form vagina of mares of the first two groups, Bifidobacterium was not found in 9-12 years old mares.

Vaginal lavage of gynecologic sick mares contained globulous microflora from groups of micrococci, i.e., Sarcina (50%). Three samples included Diplococcus, Streptococcus and Staphylococcus. From streptococci 16% of samples had α-streptococcus and γ-streptococcus.

Escherichia coli was reported in 66% of samples. From opportunistic pathogenic microorganisms four animals had sporogenous bacteria of the genus *Bacillus* (66%) and from pathogenic microorganisms 5 mares had yeast and mold fungi (83%) and four animals had thread bacteria (66%).

In the research, researchers conducted bacteriological research of vaginal blenna of mares after colting and its results are presented in Table 3.

Table 3 shows that during the postnatal period sporogenous bacteria of the genus *Bacillus* prevailed in 70 mares with the normal course of patrimonial process, considerably conceded with lactic-acid bacteria and enterococcus (Fig. 15). In the Ist days after colting bifid bacterium were recorded in small amount. Continuous presence of representatives of opportunistic pathogenic microflora presented by large number staphylococci was noted either. Yeastlike were inoculated from vaginal blenna in the first 3 days and mold fungi of the genus *Aspergillus* in 10 days after colting.

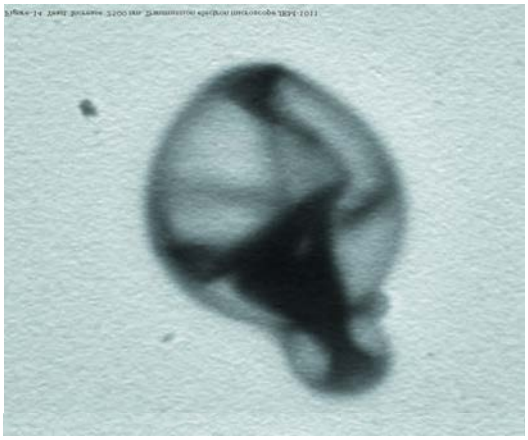

Fig. 14: Yeast increase 2500 nm; transmission electron microscope JEM-1011

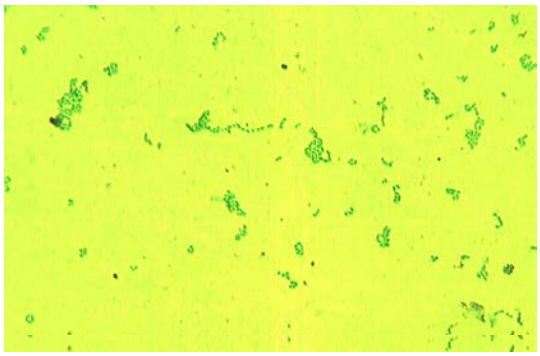

Fig. 15: Enterococcus increase x100 digital binocular biological microscope of motic BA 200

Table 3: Findings of research of vaginal microbiocenosis of mares after colting

	Age of mares (No. of animals)					
	3-5 years (54)		6-8 years (58)		9-12 years (45)	
Microorganisms	No. of animals	Percentage	No. of animals	Percentage	No. of animals	Percentage
Lactobacterium	13	52	23	82	9	52
Bifidobacterium	18	72	20	71	7	41
Sarcina	14	56	16	57	9	52
Diplococcus	13	52	18	64	11	64
Enterococcus	23	92	24	85	14	82
Streptococcus	20	80	22	78	12	70
α-streptococcus	-	-	2	7	1	5
γ-streptococcus	-	-	2	7	1	5
Staphylococcus	21	84	23	82	13	76
γ-staphylococcus	2	8	4	14	2	11
Escherichia	21	84	22	78	14	82
Sporogenous bacteria of the genus *Bacillus*	17	68	20	71	11	64
Yeast	20	80	23	82	14	82
Mold fungi of the genus *Aspergillus*	19	76	25	89	15	88

Vaginal lavage of mares of age group 4-5 years contained non-pathogenic microorganisms, i.e., Lactobacterium (52%) and Bifidobacterium (72%) and 56% of samples included bacteria of the genus *Sarcina* being one of the main representatives of microflora of vaginal blenna of mares. From families of cocci in 52% of samples were recorded Diplococcus, in 92% of samples Enterococcus, in 80% of samples Streptococcus and in of samples 84% Staphylococcus as well as 8% of γ-staphylococcus. *Escherichia coli* was reported in 84% of samples.

In 68% of samples were reported opportunistic pathogenic sporogenous bacteria of the genus *Bacillus* and from pathogenic microorganisms in 80% of samples were found yeast and in 76% of samples mold fungi.

In comparison with data of animals of the first group, vaginal lavage of 6-8 years old mares contained more Lactobacterium by 30%, Diplococcus by 12% and mold fungi by 13%. Levels of Bifidobacterium, Sarcina, Streptococcus, Staphylococcus, yeast were almost identical in comparison with indicators of the first group.

Enterococcus was reported in 24 and 2 animals had α-streptococcus, 2 animals had γ-streptococcus, 4 animals had γ-staphylococcus. Twenty two mares had *Escherichia-coli* bacteria. From opportunistic pathogenic microorganisms twenty animals had sporogenous bacteria of the genus *Bacillus* and from pathogenic microorganisms 23 mares had yeast and mold fungi and 25 animals had thread bacteria.

During investigation of vaginal lavage of clinically insane mares of age group 9-12 years 52% of samples included non-pathogenic microorganisms, namely Lactobacterium and 41% of samples contained Bifidobacterium. In 52% of samples was found globulous microflora from groups of micrococci, i.e., Sarcina. From families of cocci, in 64% of samples were found Diplococcus, in 82% of samples Enterococcus, in 70% of samples Streptococcus including in 5% α and γ-streptococcus, in 76% of samples Staphylococcus and in 11% of samples γ-staphylococcus. *Escherichia coli* (82%) was found in 14 mare. In 64% of samples were recorded sporogenous bacteria of the genus *Bacillus* and from pathogenic microorganisms, yeast (82%) was found in 15 mare including mold fungi (88%).

Thus, in farms where research was conducted, the wide spread are diseases of reproduction organs which certainly have an effect on reproductive function of mares. Mares are the main reservoir of infection secreting microbes in environment with uterus fluxes. The carriage of bacteria by healthy animals constitutes considerable danger in emergence of gynecological diseases.

DISCUSSION

As a result of bacteriological research of vaginal microflora of mares it was found that microbiological landscape is diverse. Clinically healthy mares with the normal course of birth and postnatal period the vaginal microflora is stocked with different types of non-pathogenic and opportunistic pathogenic microorganisms.

Findings of research show that vaginal medium in normal condition of many healthy mares contains non-pathogenic microorganisms stocked with corynebacteria, lactic-acid bacteria and Sarcina, partially representatives of the geni staphylococcus and streptococcus. The results of the research show that the vagina microbiocenosis of mares changes with age that is if vaginal media of mare of 3-5 and 6-8 age groups is stocked with the greatest amount of non-pathogenic microorganisms and vaginal media of mares of 9-12 age group includes the smallest amount of non-pathogenic microorganisms thus opportunistic pathogenic bacteria prevailed. Decrease in amount of lactic-acid bacteria, living in mucous membrane of vagina, promotes development of pathological changes and inflammatory processes in vagina. With age reproductive function of animals is subject to distortion and opportunistic pathogenic microflora is generally recorded in genitals.

Opportunistic pathogenic and pathogenic microorganisms prevail in vaginal blenna of mares with clinical symptoms of gynecological diseases (streptococci, staphylococci, diplococci, Escherichia, Sarcina and mold fungi), complicating the course of pathological process and reducing reproductive ability of mares. The research confirm the earlier findings by Redaelli and Codazza (1977), Blue (1987), Le Blanc (1997) and Albihn *et al.* (2003) that changes of bacterial microflora of genitals refer to the most encountered symptoms of gynecological pathologies. Researchers consider the normal vaginal microflora protects genitals from potentially pathogenic bacteria, competing for nutrient substances. During gynecological pathologies researchers observed the growth of anaerobic microorganisms. Therefore, it is possible to conceive that with excessive growth of anaerobic microorganisms various biological products are formed leading to distortion of barrier function of mucous membrane of vagina and reinforced formation of vaginal discharge.

According to Kwon *et al.* (2012) during mare endometritis considerable amount of *E. coli* was recorded in uterus. However, these data are disproved by Gunay *et al.* (2010) who denies effect of uterus microflora of mares on vaginal microflora. Nevertheless based on the results of the research, 57 clinically sick mares have *E. coli* (Escherichia) which is indicative of inflammation processes in genitals.

Albihn *et al.* (2003) highlights finding of the greatest amount of *E. coli* in uterus of mares and α-globulicidal streptococci during endometritis. In the research α and γ-streptococci were found both in healthy and clinical sick mares. Based on these findings it is possible to draw a conclusion that microbial landscape of uterus and vagina is not always similar.

CONCLUSION

Results of these research will form a basis for development of effective measures of treatment and prevention of mare infertility.

REFERENCES

Albihn, A., V. Baverud and U. Magnusson, 2003. Uterine microbiology and antimicrobial susceptibility in isolated bacteria from mares with fertility problems. Aeta. Vet., 44: 121-129.

Blue, M.G., 1987. Mycotic endometritis in mares review and clinical observations. New Zealand Vet. J., 35: 181-183.

Boone, D.R., R.W. Castenholz, G.M. Garrity, D.J. Brenner, N.R. Krieg and J.T. Staley, 2005. Bergey's Manual of Systematic Bacteriology, Volume 2. 2nd Edn., Springer, New York, USA., pp: 764-799.

Gunay, U., K. Onat, A. Gunay and M. Ulgen, 2010. Vaginal, cervical and uterine bacterial flora at the different stages of the reproductive cycle in ovariohysterectomized bitches. J. Anim. Vet. Adv., 9: 478-481.

Kwon, D.Y., S.K. Choi and G.J. Cho, 2012. Effect of uterine bacteriology and cytology on fertility in thoroughbred mares. Agric. J., 7: 245-249.

Le Blanc, M.M., 1997. The equine endometrium and the pathophysiology of endometritis. Proc. Reprod. Pathol., 1997: 78-84.

Redaelli, G. and D. Codazza, 1977. The incidence, pathogenicity and pathology of bacterial and fungal species in the mare's uterus. Folia Vet. Latina, 7: 198-204.

Reid, G., 1999. The scientific basis for probiotic strains of *Lactobacillus*. Applied Environ. Microbiol., 65: 3763-3766.

Ricketts S.W., A. Young and E.B. Medici, 1993. Uterine and Clitorial Cultures. In: Equine Reproduction, McKinnon, A.O. and J.L. Voss (Eds.). Lea and Febinger, Philadelphia, USA., pp: 234-245.

Shin, S.J., D.H. Lein, A.L. Aronson and S.R. Nusbaum, 1979. The bacteriological culture of equine uterine contents, *in vitro* sensitivity of organisms isolated and interpretation. J. Reprod. Fert. Suppl., 27: 307-315.