eISSN: 09748369

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Biology and Medicine

Research Article

Volume 6, Issue 4, Article ID: BM-052-14, 2014

Indexed by Scopus (Elsevier)

www.biolmedonline.com

Vaginal microbiocenosis of mares bred by stallions after vasectomy

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Citation: Mardanovna JN, Kenesovna TZ, Nurmuhambetovich JM, Orynbay T, Erbolatovna AM, *et al.* (2014) Vaginal microbiocenosis of mares bred by stallions after vasectomy. Biol Med 6(4), Article ID: BM-052-14, 5 pages.

Received: 3rd Dec 2014; Accepted: 30th Dec 2014; Published: 10th Jan 2015

Abstract

We carried out studies to determine microbial vaginal flora during prevention of genital infantilism at mares. The aim of this work was to study the effect of stallions after vasectomy on vaginal microbiocenosis of mares. We used generally accepted methods of bacteriological studies and electron microscopy. Vaginal flora of mares is characterized by nonpathogenic and opportunistic microorganisms. Lactic acid bacteria, lactobacilli, bifidobacteria, and sarcines were identified most commonly. No pathogenic microflora was detected in vaginal swabs. Nonpathogenic microorganisms were from the following classes: *Corynebacterium*, *Bifidobacterium*, *Diplococcus*, *Streptococcus*, *Staphylococcus*. No pathological changes in the vagina of mares were detected. We did not find any significant differences in vaginal microbiocenosis of mares with and without systematic sexual contact with stallions after vasectomy.

Keywords: *Lactobacterium*; *Bifidobacterium*; *S*arcina; *Corynebacterium*; *Diplococcus*; *Streptococcus*; *Staphylococcus*.

Introduction

Veterinary medicine and practice pay a great attention to the problem of infertility of farm animals. However, distribution and economic damage caused by infertility remains unchanged. For this reason, the search for safe and effective methods of treatment and prevention of gynecological pathology is an urgent task of the veterinary medicine. This is particularly important in the sports horse breeding where exploitation conditions and sharply continental climate cause frequent and sever abnormalities that hinder reproduction of animals.

Microbiocenosis of genitals of mares is of utmost importance for the development of prevention and treatment of reproductive system.

Different populations of microorganisms circulate in all horse-breeding farms. These populations of microorganisms may be atypical, i.e., modified under the influence of a number of factors, for example, under the influence of antimicrobial medicines, transferring during gynecological examination, mating, and others. According to several authors [1-4], microorganisms can cause various adverse changes throughout the body including the reproductive system.

According to some scholars changes in bacterial microflora of genitals are the most common signs of reproductive disorders [5-8]. Therefore, the study of vaginal microbiocenosis of mares bred by stallions after vasectomy is a relevant problem.

Materials and Methods

We have carried out our studies during the period of growth and development of young replacement. Vaginal swabs of mares were collected for bacteriological examination. Mares were divided

into two groups (experimental and control). The first group consisted of mares that had systematic sexual contact while breeding with stallions after vasectomy. The second group consisted of mares that had no sexual contacts with stallions.

Upon the collection of vaginal swabs of mares we strictly observed basic principles of asepsis and antisepsis. Vaginal swabs were obtained using the device for pig insemination – "POS-5" (made in Russia). One bottle of "POS-5" device contained 50 ml of sterile saline.

Before vaginal swab collection, we have carried out the toilet of the external genitalia of mares with the solution of furacilinum 1:5000 followed by drying them with dry paper towel. After swab collection they were placed in an incubator with ice and transported to the laboratory for testing.

When performing this work, we used conventional methods of bacteriological studies [9]. In order to get the culture of microorganisms from vaginal swabs, we made four successive sets of dilution in tubes (1:10, 1:100, 1:1000, 1:10000) with a sterile saline solution.

Samples from swabs were plated on meat-peptone agar, MRS AGAR (DE MAN, ROGOSA, SHARPE) – special medium for lactic acid bacteria, nutritive medium of milk hydrolyzate, HMM for bifidobacteria, Buchin's medium for corynebacteria culturing, medium containing sodium azide – for *enterococci*, Baird-Parker's medium – for *staphylococci*, wort agar, Sabouraud medium – for fungi, Endo agar, Bismuth sulfite agar – for enterobacteria. We also studied the ability of microorganisms to sporulation. Counting of colonies was performed on colony counter. Smears were prepared from overnight cultures, Gram staining and examined under the microscope.

By the way we studied culture-morphological characteristics of microorganisms by plating them on meat-peptone broth, agar, and gelatin. Biochemical activity of cultures was studied by plating on Hiss meduim. Biochemical properties were determined by the ability of enterobacteria to ferment carbohydrates. Identification of microorganisms and their taxonomic distribution was conducted in accordance to Bergey's manual of determinative bacteriology [10,11].

Petri dishes with bacterial inoculation were incubated at 37°C for 48 h. Typical single colonies of microorganisms were subcultured into tubes with plain agar and nutritive medium for differential diagnostic. Each colony was inoculated into 3 vials and placed in an incubator at 37°C. After 48 h we observed growth of pure cultures.

Preparation of material for electron microscopy was performed according to the conventional method [12].

In laboratory studies, we used a steam sterilizer (autoclave) VK-75-01 OKP 945121000206 made in Russia, drying cabinet SH-80-01 SPU TU 9452-010-00141798-2005 made in Russia, electric dry-air thermostat TSO-1/80 SPU TU 9452-004-00141798-2000 made in Russia, heated bath – made in Russia, transmission electron microscope LEM-1011 equipped with a digital CCD camera Morada (OLYMPUS) JEOL made in Japan and Digital Binocular Biological Microscope Motic BA 200 made in Austria.

Results

In order to determine the degree of influence of stallions after vasectomy on the vaginal microflora, we studied vaginal microbiocenosis of mares with and without sexual contact with stallions after vasectomy. Thereto we formed two groups of 21 mares. The first group consisted of mares that had systematic sexual contact with stallions after vasectomy. Smears for biological studies were collected from the vagina of mares in the stage of equilibration of the sexual cycle.

Vaginal swabs were studied from 42 mares in the breeding season, during pregnancy and in the postpartum period. The results indicate that both groups of mares had pathogenic and opportunistic microorganisms. The results of these studies are presented in the Table 1.

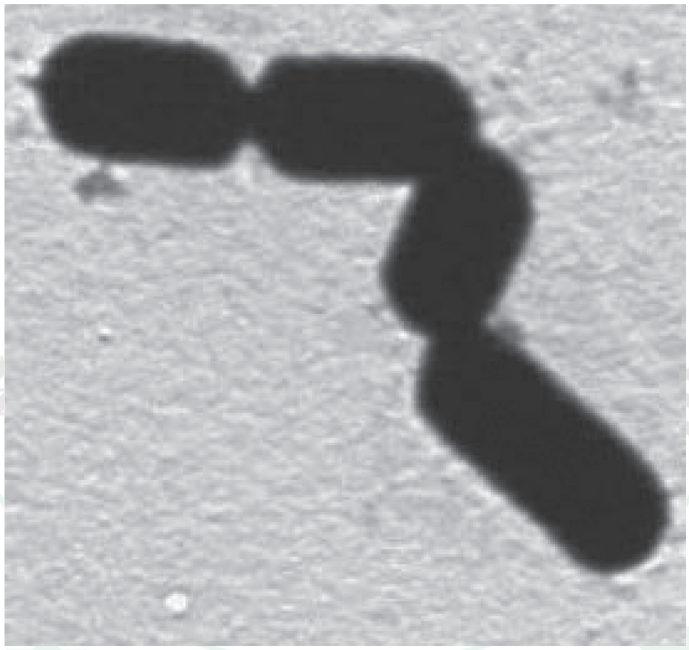
As seen from the Table 1, lactic acid bacteria were the most frequently isolated microorganisms in all groups of mares (*Lactobacterium* – 100%) (Figure 1) which in turn have been subdivided into the following species: *Lactobacillus acidophilus*, *Lactobacterium casei*, *Lactobacterium plantarum* by morphological, cultural, and physiological characteristics. The main representatives of vaginal microbiocenosis of mares were bifidobacteria and sarcines.

Bifidobacterium was identified in 61.9-76.2% of the samples. Moreover, the number of bifidobacteria was considerably higher in the

Table 1: Vaginal microbiocenosis of healthy mare.

Identified microflora	Groups			
	Experimental, $n = 21$		Control, $n = 21$	
	N	%	N	%
Lactobacterium	21	100	21	100
Bifidobacterium	16	76.2	13	61.9
Sarcina	17	80.9	16	76.2
Corynebacterium	15	71.4	16	76.2
Diplococcus	7	33.3	5	23.8
Streptococcus	6	28.6	5	23.8
α-*Streptococcus*	3	14.3	2	9.5
γ-*Streptococcus*	1	4.8	3	14.3
Staphylococcus (γ-*staphylococcus*)	4	19.0	3	14.3

Figure 1: *Lactobacterium*: increase 8000 nm. Transmission electron microscope JEM-1011.



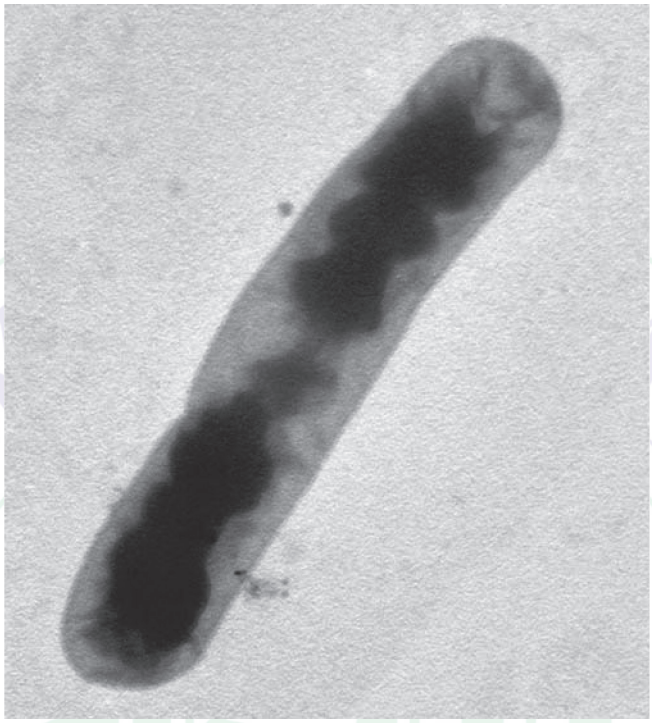
vaginal smears of mares of the experiment group (76.2%) than that in the control one (61.9%).

Sarcina, a spherical bacterium from micrococci group, was identified in 76.1-80.9% of vaginal swabs smears of mares. The number of sarcines was slightly higher in mares of the experimental group (80.9%) as compared with the control group (76.2%).

We did not identify pathogenic microflora in vaginal smears of animals of the experimental and control groups. The identified opportunistic microorganisms belonged to the classes of *Corynebacterium* (71.4-76.2%), *Bifidobacterium* (61.9-66.6%). Corynebacteria (Figure 2) were detected in 71.4% of samples of the experimental group which is less by 4.8% than in the samples of control animals.

Cocci were presented by non-pathogenic cultures – classes *Diplococcus* (23.8-33.3%), *Streptococcus* (23.8-28.6%), including α-streptococcus (9.5-14.3%), γ-streptococcus (4.8%) and γ-staphylococcus (14.3-19.0%). It should be also noted that diplococci were identified in higher proportion in vaginal smears of

Figure 2: *Corynebacterium*: increase 12,000 nm. Transmission electron microscope JEM-1011.



animals of the experimental group as compared to the control one – 9.5%.

Also streptococci and staphylococci were identified in a higher proportion in samples of mares of experimental group – 28.6% and 19.0% respectively, as compared to the control group. The differences in both groups were insignificant.

Data analysis of the bacteriological research shows that the allocation of α-streptococcus from vaginal smears of mares of the experimental group was considerably higher than from the smears of mares in the control group (4.8%).

The number of γ-streptococcus in vaginal swabs from mares of experimental group was similar to the α-streptococcus of mares of the control group. However, this value was higher by 9.5% compared with the experimental group.

Identification of γ-staphylococcus in the swabs of mares of experimental group was by 4.7% higher than that in the control group. This difference was not significant.

Isolation of α-streptococcus in vaginal swabs of mares of the experimental group was considerably higher than that in samples of mares of the control group (4.8%).

Discussion

It is well known from the literature that all the natural cavities of the body contain micro-organisms maintaining their normal functional state [1-4]. Our studies confirm this fact.

In mares with and without systematic sexual contact with stallions after vasectomy vaginal flora was inhabited by various species of nonpathogenic microorganisms. Moreover, in the mares during oestrus without systematic sexual contact with stallions after vasectomy vaginal microflora was presented by a slightly lower number of species as compared to that of the mares that had sexual contact with stallions after vasectomy.

Apparently, a slight increase in the amount of vaginal microflora in mares that had sexual contact with stallions after vasectomy was caused by their penetration from the external environment during mounting of stallions. We believe that the sufficient number of such microflora did not allow the growth and development of newly arrived microorganisms.

It is known that lactic acid bacteria have adhesive properties which protect mucosal epithelium of the vagina from other microorganisms [5,7,9]. Besides, the normal vaginal microflora

protects vagina from potentially pathogenic bacteria by competing for nutrient medium. Due to this fact pathological process in the vagina of mares did not develop.

Vaginal medium of mares had the following nonpathogenic microorganisms: *Lactobacterium*, *Bifidobacterium*, Sarcina, *Corynebacterium*, partly representatives of the genus *Diplococcus*, *Streptococcus*, and *Staphylococcus*.

Conclusion

In the vagina of mares there are different types of non-pathogenic microorganisms that protect it against potentially pathogenic bacteria.

Protection is carried out by the adhesive properties of bacteria and their competition for nutritious medium.

There is no significant data that the use of stallions after vasectomy affects the vaginal microbiocenosis in mares. Sufficient amount of beneficial microflora of the vagina does not allow the development of microorganisms entering during the coitus.

The main inhabited species of vaginal environment of mares are *Lactobacterium*, *Bifidobacterium*, Sarcina, *Corynebacterium*, partially *Diplococcus*, *Streptococcus and Staphylococcus*.

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