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MYCOPLASMA CONTAMINATION OF BULL SEMEN USED FOR ARTIFICIAL INSEMINATION

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Technologies of artificial insemination of cattle are actively used in livestock farms in Russia. At the same time, quality control of semen production does not involve testing the bull semen for microorganisms of the genus Mycoplasma. The active use of molecular genetic methods allows for the rapid determination and species identification of pathogenic mycoplasmas; however, it does not indicate the viability of the identified pathogen. The paper aims to study mycoplasma contamination of semen products presented on the Russian market and determine the conformity of the results of the Polymerase Chain Reaction [PCR] method and the traditionally used microbiological method. The paper presents the results of the first Russian study of the occurrence and viability of pathogenic species Mycoplasma bovis, M. bovigenitalium, M. californicum, and Ureaplasma diversum in semen production. We investigated 447 samples of cryopreserved semen of bulls-producers of various breeds from Russian breeding centers and breeding farms of the USA, Great Britain, and the Netherlands, supplying these products to the Russian market. The analysis shows a high frequency of occurrence in the bull semen of DNA of microorganisms of the genus Mycoplasma (up to 70.9%). Besides, M. bovigenitalium appeared to be the most frequently detected type of mycoplasma from those tested. Cases of identifying several types of mycoplasmas in one sample were revealed: in 58.8% of the studied samples of semen products from Russian farms and 15% of semen samples from international breeding centers. The viability of mycoplasmas that were positive according to PCR analysis when testing 65 samples was demonstrated using the microbiological method for 94% of samples. The correspondence of the results of microbiological and molecular genetic detection of mycoplasmas and ureaplasmas was 88.3%. The results obtained indicate the prevalence of mycoplasmas in semen production and the need to improve the control system of breeding material in order to prevent the spread of mycoplasma infections of cattle using artificial insemination technologies.

Keywords: *Mycoplasma spp; polymerase chain reaction; bull semen; microbiological method; artificial insemination*

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МИКОПЛАЗМЕННАЯ КОНТАМИНАЦИЯ СПЕРМЫ БЫКОВ, ИСПОЛЬЗУЕМОЙ ДЛЯ ИСКУССТВЕННОГО ОСЕМЕНЕНИЯ

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Технологии искусственного осеменения крупного рогатого скота активно применяются в животноводческих хозяйствах России, при этом контроль качества спермопродукции не предполагает тестирование спермы быков на наличие микроорганизмов рода Mycoplasma. Активное применение молекулярно-генетических методов позволяет проводить быстрое выявление и видовую идентификацию патогенных микоплазм, однако не говорит о жизнеспособности выявленного патогена. Целью работы являлось изучение микоплазменной контаминации спермопродукции, представленной на рынке Российской Федерации и определение соответствия результатов метода ПЦР и традиционно применяемого микробиологического метода. В работе приведены результаты первого для России исследования встречаемости и жизнеспособности патогенных видов Mycoplasma bovis, M. bovigenitalium, M. californicum и Ureaplasma diversum в спермопродукции. Исследовано 447 образцов криоконсервированной спермы быков-производителей различных пород из отечественных племенных центров и племенных хозяйств США, Великобритании, Нидерландов, поставляющих данную продукцию на рынок Российской Федерации. Проведенный анализ показал высокую частоту встречаемости в сперме быков ДНК микроорганизмов рода Mycoplasma (до 70, 9%), M. bovigenitalium была наиболее часто выявляемым видом микоплазм из тестируемых. Показаны случаи идентификации сразу нескольких видов микоплазм в одном образце – в 58,8% исследованных образцов спермопродукции из отечественных хозяйств и в 15% образцов спермы из зарубежных племенных центров. Жизнеспособность микоплазм, положительных по данным ПЦР-анализа, при тестировании 65 образцов, продемонстрирована микробиологическим методом для 94% образцов. Соответствие результатов микробиологического и молекулярно-генетического обнаружения микоплазм и уреаплазм составило 88,3%. Полученные результаты свидетельствует о

распространенности микоплазм в спермопродукции и необходимости совершенствования системы контроля племенного материала с целью предупреждения распространения микоплазменных инфекций КРС при использовании технологий искусственного осеменения.

Ключевые слова: *Mycoplasma spp.; PCR; сперма быков; микробиологический метод; искусственное осеменение*

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Introduction

Currently, breeding products are the subject of active international trade. Deliveries of bull semen for artificial insemination to Russia are growing annually. The leading exporters of breeding material are the USA, Canada, Denmark, and Germany. Russian breeding centers are also engaged in selling semen products, focusing on Russian farms and farms of Belarus, Azerbaijan, Kazakhstan, and Uzbekistan. With all the indisputable advantages of using cryopreserved bull semen, artificial insemination technology does not exclude the risks of spreading infectious diseases. Frozen semen is an ideal system for preserving the viability of microorganisms. To assess the quality and safety of bull semen in Russia, studies are conducted under the state standard, according to which the total presence of pathogenic and conditionally pathogenic bacteria and fungi is determined in semen doses. However, methods for detecting viruses, protozoa, and mycoplasmas are not included in this document. At the same time, the transmission of mycoplasmas through semen intended for artificial insemination is characterized by a high degree of risk [9; 11]. Previously, Mycoplasma bovis, ingested with infected sperm, was shown to cause lesions of the reproductive tract of cows [10]. The isolation of mycoplasmas with semen often proceeds without clinical manifestations in a donor bull [12]. Furthermore, cases of M.bovis and other Mollicutes that cause diseases of cattle (M. bovigenitalium, M. californicum and Ureaplasma diversum) often go unnoticed, being one of the causes of diseases of calves and the development of mastitis in cows [9].

The microbiological method is considered a classical method of identifying mycoplasmas [14]. However, specialized media and certain conditions are necessary for the growth of mycoplasmas [13]. At the same time, the growth of other bacterial species often significantly complicates or makes it impossible

to identify and differentiate mycoplasmas [3]. The study devoted to detecting M. bovis revealed that cultivation is characterized by a sensitivity that does not exceed 75% [6].

To detect mycoplasmas in various biological materials, the Polymerase Chain Reaction [PCR] method is widely used, which allows one to detect mycoplasmas with high sensitivity and determine their species [5; 7; 8; 15; 16]. However, the PCR method does not allow one to judge the viability of microorganisms found in the sample, which is essential for assessing the safety of bull semen used for artificial insemination.

The paper presents the results of studying the prevalence of M.bovis, M. bovigenitalium, M. californicum, and Ureaplasma diversum in the semen production of cattle of Russian and international breeding farms and evaluating the possibility of using molecular genetic and microbiological methods to assess the quality and safety of genetic material used for artificial insemination.

Materials and methods

We examined 447 samples of semen doses of various bulls from Russian meat and dairy farms and Holstein bulls from breeding centers in the UK, the Netherlands, and the USA. The first research task was to identify the genetic material of mycoplasmas by PCR and assess the viability of mycoplasmas using the microbiological method. The second task was to determine the correspondence of the results obtained using different methods.

The detection of Mycoplasma microorganisms in semen doses was performed using a PCR kit with electrophoretic detection of amplification products "MIK-KOM" (Amplisens, Russia). Identification of M. bovis, M. bovigenitalium, M. californicum, and U. diversum species was carried out by PCR with hybridization-fluorescence detection of amplification products on the Rotor Gene Q device using previously developed methods [1]. To confirm the results obtained, we also used the LSI VetMAX™ Mycoplasma bovis PCR kit (France).

The viability of mycoplasmas in cattle semen was confirmed for 65 samples of bull semen, in which the DNA of Mycoplasma microorganisms was detected using PCR. Cultivation was carried out on specific media according to the method of M. Ogata [13] with modifications [3]. Each sample was cultivated on four media with the addition of glucose, L-arginine, a mixture of glucose, L-arginine, and urea. The duration of cultivation was three weeks. After considering the cultivation results, 43 cultures were additionally tested using PCR for the presence of DNA of Mycoplasma microorganisms without species

differentiation to assess the correctness of the interpretation of the results obtained and determine the presence of M. bovigenitalium, M. californicum, M. bovis, and U. diversum species.

Results

Thus, PCR studies of 447 semen samples obtained from bulls of various breeds showed that the DNA of microorganisms of the genus Mycoplasma was detected in 70.9% of the samples studied (Table 1).

Table 1.

Results of detecting mycoplasma DNA in semen samples

Pathogen	Bull semen from Russian breeding centers		Bull semen from international breeding centers		Total	
	214 samples		233 samples		447 samples	
	DNA detected	%	DNA detected	%	DNA detected	%
Mycoplasma spp.	183	85.5	134	57.5	317	70.9
M. bovigenitalium	129	60.3	52	22.3	181	40.5
M. californicum	109	51.7	46	19.7	155	34.9
Ureaplasma diversum	116	55	24	10.3	140	31.5
M. bovis	0	0	7	3	7	1.6

In bull semen from Russian breeding centers, mycoplasma DNA was found almost twice as often as in imported semen products: in 85.5% and 57.5%, respectively. M. bovigenitalium DNA was detected in 60.3% of Russian and 22.3% of imported semen samples, M. californicum DNA was detected in 51.7% and 19.7% of samples, respectively. The DNA of Ureaplasma diversum was found in 55% of semen samples of bulls from Russian breeding centers and in 10.3% of sperm samples of foreign origin.

Besides, M. bovis DNA was found only in semen samples of bulls from breeding centers in the USA. Testing of samples using the LSI VetMAX™ Mycoplasma bovis test system confirmed the results obtained.

Semen coinfection with various types of mycoplasmas was observed in 124 samples from Russian farms (58.8%) and in 35 samples of semen from international breeding centers (15%). Coinfection of M. californicum/M. coinfection (98 cases, 22.1%) and M. californicum/M. bovigenitalium (86 cases, 19.4%) were most frequently detected. Simultaneous infection of M. bovigenitalium, M. californicum, and U. diversum was observed in 52 samples

(24.6%) of semen from Russian farms and in 4 samples (1.7%) of semen from international breeding centers.

For 61 out of 65 samples of bull semen positive according to PCR (94%), growth was recorded on selective nutrient media. The correspondence of the results of microbiological detection and the results of DNA detection of Mycoplasma microorganisms without differentiation of the type of microorganisms in the obtained cultures was 88.3%. When testing the obtained cultures by PCR, M. bovigenitalium and M. californicum were detected in 12 (27.9%), and 7 (16.2%) of the 43 cultures studied. Ureaplasma diversum was also found in 16.2% of cultures. In 4 out of 43 cultures, the presence of several types of mycoplasmas was confirmed. M. californicum and U. diversum were recorded in one culture, M. genitalium and U. diversum in another, and M. bovigenitalium, M. californicum and U. diversum were simultaneously detected in two more cultures.

Discussion

The role of mycoplasma infection in the development of diseases of the reproductive organs of cattle has been discussed in various scientific papers since the 1960s [2]. Currently, the problem still remains relevant worldwide due to the lack of adequate control of semen production. For instance, in 2018, the results of a study of an outbreak of mastitis in cows in Finnish farms were published, according to which the cause of the disease was semen used for artificial insemination of cows contaminated with mycoplasmas [10].

In our study, M. bovis was detected only in samples from international breeding farms, whereas a publication of 2020 reported that this microorganism was detected in 11.6% of semen doses from Russian breeding farms [4]. Such differences may be related to the different levels of control over the health of bulls in different breeding farms that supply semen for artificial insemination. At the same time, as in another paper [4], our study shows that M. bovigenitalium is the most common mycoplasma pathogen in semen production.

In previous studies comparing molecular biological and microbiological methods for detecting mycoplasmas, a sufficiently high degree of consistency of results for both methods in the study of different types of biological material was shown [14]. In our paper, cultivation was carried out only for samples in which the presence of DNA of Mycoplasma microorganisms was confirmed. For these samples, a high level of compliance with the results of microbiological and molecular genetic methods was demonstrated. In another paper [14], when examining sperm for the presence of mycoplasmas, 50% of the studied samples

were positive both as a result of a PCR study and according to cultivation data. Such differences may be associated with both a violation of the storage and transportation conditions of samples, which led to a decrease in the viability of mycoplasmas and a low content of microorganisms in semen. In general, the results obtained confirm the possibility of detecting several types of mycoplasmas in biological material at once [14].

Conclusion

The obtained results of the study of sperm samples using PCR techniques and microbiological method confirm the possibility of transmitting mycoplasmas, including pathogenic species M. bovis, M. bovigenitalium, M. californicum, and U. diversum, during artificial insemination. A high degree of mycoplasma infection was revealed in the studied samples of bull semen used for artificial insemination. We discovered that a significant part of mycoplasmas in semen production (up to 94%) retains its viability.

The data obtained confirm that molecular biological and microbiological methods in the study of semen production of cattle for the presence of mycoplasmas complement each other.

The obtained results emphasize the insufficiency of the methods used to control the safety of semen products entering the Russian market and the relevance of screening cattle sperm products aimed at detecting Mycoplasma bovis, Mycoplasma bovigenitalium, Mycoplasma californicum, and Ureaplasma diversum to confirm the quality of bull semen and prevent the spread of mycoplasma infection.

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