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19th INTERNATIONAL CONGRESS ON BIOTECHNOLOGY IN ANIMAL REPRODUCTION (ICBAR)


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REGULATORY COMPLIANCE ABOUT THE SANITARY AND QUALITY CONDITIONS OF BULL SEMEN IN WEST BALKAN: ARE WE CLOSER OR FURTHER AWAY FROM THE EUROPEAN UNION?

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Abstract: Diagnostic and health condition control procedures of bulls in the semen collection centres in Serbia are in accordance with regulation on the establishment of animal health care measures for 2015, which was published by Serbian Ministry of Agriculture. Regarding the requirements for the health inspection of the bulls in Serbia, Bosnia and Herzegovina and the European Union (EU), there are certain differences. For example, serological tests are performed on each breeding bull in Serbia twice a year (in the EU 1x year), especially tests for *enzootic bovine leukosis*, *bovine virus diarrhoea* and *infectious bovine rhinotracheitis/infectious pustular vulvovaginitis*. Regulatory compliance in Bosnia and Herzegovina differs in certain extent from one in Serbia. As an example of requirements in terms of distribution of semen in the EU provided in this work are provisions of Directive 88/407/EWG. Frozen semen of bulls should meet the following minimum quality requirements for the ability for fertilization: progressive motility of spermatozoa at least 50% and percentage abnormal spermatozoa morphology up to 30%. Apart from everything, there is a condition that one dose contains at least 10 million spermatozoa after thawing and Mueller resistance test which requires minimum 40% of the progressive motile spermatozoa. On the other side, there are Recommendations of the *Arbeitsgemeinschaft Deutscher Rinderzüchter* (2006) about requirements for breeding bulls which are used for artificial insemination and natural mating. It is just required that two-thirds of the obtained ejaculate, after thawing should show a progressive sperm motility forward of at least 50%.

Having in mind differences in subsidiary legal acts, in terms of bull’s health, which are applicable in Serbia, Bosnia and Herzegovina in relation to the Example from the EU, we believe that their adaptation is necessary. Nevertheless, regulations related to semen quality ought not to be altered until the Serbian entrance to the EU. We believe that there would exist certain confusion among clients having the present market conditions in mind in case of allowing free distribution without any quality control.

Key words: bull, Germany, Serbia, legislative, sperm

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Introduction

Development of artificial insemination (AI) along with embryo cryopreservation has led to large scale exchange of cattle germplasm over the past 60 years, thus taking advantage of financial, sanitary, and animal welfare aspects compared to movement of live animals (Ponsart and Pozzi, 2013). As experimental data show that many animal pathogens can be associated with semen and embryos (Bielanski, 2006; Van Soom et al., 2010) the basic principle to ensure a high level of biosecurity for semen relies on the concept of pathogen-free semen collection centre (Thibier and Guerin, 2000). The market of semen was changed in recent decades due to the development of cattle breeding (genomic selection) and the process of globalization. Veterinary profession should continue to play a significant role in the process of production, trade and application of semen for artificial insemination, despite the fact that farmers take on more routine activities in breeding cattle and livestock in general. They are, for example, artificial insemination and certain forms of therapy in the stable (formally under the supervision of a veterinarian). This article will focus on the regulatory compliance about the sanitary and quality conditions of bull semen in some countries of West Balkan. Variations between sanitary and semen quality requirements in the West Balkan and the European Union (for example in Germany) will be described as well as possible consequences on trade of semen.

Diagnostic and health conditions

Semen collection centre, which wants to trade inside the EU, has to be registered accordingly to appointments of Directive 88/407/EWG (Anonymous, 1988) and following regulations. Furthermore, there are obligations of serology diagnostic tests of bulls on: brucellosis, infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) and enzootic bovine leucosis (EBL). This applies for bulls in the following cases: in period of 28 days before movement to quarantine of semen collection centre, bulls in quarantine of semen collection centre (excl. EBL), bulls in the semen collection centre 1x per year. Related to bovine virus diarrhoea (BVD), antigenic and serologic testing is compulsory for bulls in period of 28 days before movement to quarantine of semen collection centre, bulls in quarantine of semen collection centre, serologically negative bulls in the semen collection centre 1x per year, but also control of semen from serologically positive bulls before the first posting of semen. For tuberculosis it is compulsory to provide the skin test. Diagnostic of campylobacteriosis and trichomoniasis include preputial lavage or taking lavage samples from the artificial vagina. Anyway, in Germany, it is further required that the animals meet the provisions related to contagious diseases on the domestic market, according to the Regulation on protection against infectious diseases from 22 of May 2013 (Anonymous, 2013).

Diagnostic and health condition control procedures of bulls in the semen collection centre in Serbia are in accordance with Regulation on the establishment of animal health care measures for 2015 (Anonymous, 2015), which was published by Serbian Ministry of Agriculture. Serological tests in semen collection centre are performed on each breeding animal twice a year, and those include tests for: brucellosis, EBL, BVD, IBR/IPV, leptospirosis and Schmallenberg virus. It is also compulsory to conduct tests for tuberculosis (skin test), as well as for campylobacteriosis and trichomoniasis (local diagnostic procedure). Viral examinations of bull semen using PCR method are also
conducted for: BVD and Schmallenberg virus every six months, and IBR/IPV every three months.

Among everything, semen that is allowed for importing to Bosnia and Herzegovina should be examined for brucellosis, Campylobacter fetus spp. venerealis, Trichomonas foetus, IBR/IPV. This bull semen should be accompanied by a certificate (Anonymous, 2014a), issued by authorized veterinarian. The certificate should confirm: that purebred breed bulls, which semen is taken from, are healthy and did not exhibit symptoms which allow the suspicion to infectious diseases. These relate to the kind of symptoms that are responsible for diseases transmissible by this breed of animal. Regarding contagious diseases the conditions for import into Bosnia and Herzegovina are that purebred breed bulls, which the semen originates from, are from herds that are free of many infectious agents. These are the following: Tuberculosis bovis, paratuberculosis, bovine trichomoniasis (Trichomonas foetus), bovine campylobacteriosis (Campylobacter fetus spp. venerealis), brucellosis (Brucella abortus), foot and mouth disease, EBL, BVD, pleuropneumonia contagiosa bovum, cattle plague (Pestis bovina), blue tongue disease, bovine trichophytosis and mange in cattle (Scabies). That the purebred breed bulls, which the semen is taken from, constantly are being examined clinically and are not naturally mated for at least 30 days before sampling semen. Certain laboratory tests are determined for bulls as semen donors when it comes to importing semen into Bosnia and Herzegovina. It should be the purebred bulls, which the semen is tested from and obtained negative results of the following diseases: tuberculosis – simultaneously with bovine and avian tuberculin; Trichomonas foetus; IBR/IPV; EBL, brucellosis (with the result less than 30 IJ in 1ml of semen).

However, currently there is an ongoing situation with blue tongue disease in our neighbor country Croatia. This country has been conducting planned vaccination on the Croatian territory since 02.02.2015 (Anonymous. 2014c). Those activities are officially led by Ministry of Agriculture in Croatia – Directorates for Veterinary and Food Safety. Since the disease has been established and confirmed on Croatian territories, measures taken also include vaccination of all cattle, sheep and goats which are older than three months twice in one month period. The vaccination is performed by official veterinary organizations and thus it is available and free for owners. The owners are obliged to provide veterinarians with gathered and properly marked animals prior to their arrival in order to assure provision of vaccination. Therefore, it is rather essential in epizootic terms what measures shall be taken when it comes to frozen semen along with breeding bulls originating from Croatia, meant for importation to Serbia.

**Semen quality**

As an example of a country with developed cattle breeding, there are measures in Germany which are actually recommendations about the requirement for breeding bulls in the “ADR” rules 8.2 and 8.3 (Arbeitsgemeinschaft Deutscher Rinderzüchter, 2006). In relation to the fertilizing ability in order to assess the quality of the sperm minimum requirements are evaluated in ejaculate: volume, sperm concentration (density), progressive motility and abnormal spermatozoa morphology after collection, but also progressive motility after a deep freeze. The conditions for the free trade of semen are met when all minimum criteria are fulfilled. To make a reliable assessment, three examination of the semen are required, each one conducted within 3 to 5 days (for collecting semen). Should all criteria are not satisfactory, collection will be repeated with next two ejaculates within 14 days, however not longer than 4 weeks. The minimum values for the quality of
the sperm are as following. For undiluted semen, appearance of semen: consistency: creamy to milky; color: ivory to white or yellow-white; without admixtures. Volume ought to be: for bulls to 2 years: 2.0 ml, and for bulls older than 2 years: 4.0 ml. Sperm concentration ought to be 600.000/µl, percentage of morphologically changed: less than 20%; 5% maximum with altered head and 10% changes on acrosome. Mass motion should be undisturbed and progressive motility ought to be 70% minimum. Conditions for deep-frozen semen are two-thirds of the obtained ejaculate, after thawing should show a progressive sperm motility of at least 50%.

The following conditions regarding semen quality in Serbia are defined by the Regulation about semen quality (Anonymous, 2014b). This regulation also contains details for labeling and packaging of semen doses. The introduction of the regulations defines that the semen of domestic animals is marked in a manner that prevents the replacement of doses of semen or any abuse. The name of the semen collection centre that has produced the semen, the name, breed and identification number (in the main register records) of the bull from which the semen originates and the date of issuing the semen have to be labeled on the outer surface of the straw. It is defined that the semen meets the requirements in terms of quality if: it is produced in a semen collection centre, it meets the health and hygiene conditions in accordance with recognized international standards, it comes from high in quality breeding animals for which the permit was issued for breeding. Thus is conditioned that is taken from an animal that has been clinically examined – without any symptoms of infectious diseases, from the animals with known health status over which are implemented Regulation on the establishment of animal health care measures for 2015, (Anonymous, 2015), and it meets the minimum requirements for the quality of semen. The minimum quality requirements of native bull sperm are firstly fertile ability of ejaculates for preparation of dozes and for utilization in breeding. These are the following: volume of 2 ml, ivory or yellow color, milky consistency, smell – specific for species and pH: 6.5 – 7.0. It also includes progressive motility of spermatozoa: 65% minimum; mass motion: good; density: 500x10^6/ml; percentage of morphologically changed spermatozoa: up to 20% and total number of bacteria up to do 5000 CFU (colony forming units) in 1ml. The fertile ability of diluted semen secondly, where following conditions are set: progressive motility of spermatozoa: 70% minimum; percentage of morphologically changed spermatozoa: up to 20%; number of progressive motile and morphologically normal spermatozoa in dosage for insemination: 5 million minimum; total number of bacteria up to 500 CFU/ml. Without diminishing the previous features, we believe that in practice the most important are the criteria for fertile ability of deep-frozen semen. There are the following conditions: progressive motility of spermatozoa: 50% minimum; percentage of morphologically changed spermatozoa: up to 30%; progressive motility of spermatozoa according to resistance test: 40% minimum; number of progressively motile and morphologically not altered spermatozoa in dosage for insemination: 10 million minimum; total number of bacteria up to 500 CFU/ml. Finally, the fact is emphasized regarding the minimal conditions for semen quality according to the regulations may deviate from the prescribed value when the semen is used for experimental purposes or the introduction of new insemination techniques. Furthermore, when this is a case the semen is specifically marked as semen for experimental purposes or the introduction of new insemination techniques.

According to the certificate of import of semen to Bosnia and Herzegovina (Anonymous, 2014a) conditions for semen quality are specified the semen should be taken and stored under veterinary-sanitary control and stored in liquid nitrogen. This semen
should be guarded (stored) in a suitably sealed and visibly labeled containers that have been cleaned, disinfected or sterilized, and in approved warehouses applied extenders should be sterile before adding prepared semen, frozen semen of bulls should meet the following minimum requirements for the ability for fertilization: progressive motility of spermatozoa at least 50%; percentage abnormal spermatozoa morphology up to 30%. Apart from everything, there is a Mueller resistance test which requires that one dose contains at least 10 million spermatozoa after thawing and minimum 40% of the progressive motile spermatozoa.

**Provisions of breeding bulls**

In relation to the pedigree, hereditary diseases, general health and the health of male genital tract, the provisions relate to the “requirement for breeding bulls used for artificial insemination and natural breeding” determined by the ADR rules 8.2 (2006). Furthermore, the animals need to fulfill the requirements relating to infectious diseases. Other provisions of this list of recommendations relate to **impotentia coeundi** as well as **impotentia generandi** in trade relations. When it comes to guarantee for **potentia coeundi** for the sold bull, its ability for sexual reflex to other animals is checked after at least two weeks in the new environment. The bull has at least one day after the break (not to mate him one day) to jump to a minimum of two-thirds of cows exposed to him. The reaction time for the jump is 10 minutes. Examination of the **impotentia generandi** of the sold bull, with one jump he needs to fertilize 4 of 6 cows. However, if these conditions are not fulfilled, the purchaser has the right to request examination of the sperm quality on the expense of the distributor. It is also compulsory to obtain proof about the health status of the bull genitals issued by a veterinarian. In Serbia, there is only a recommendation that the impotentia is considered as a defect in the purchase (Aleksic and Djukic, 2001). In this case, this defect relates to the law regulating obligation relations of seller and the buyer.

**Discussion and conclusion**

Based on the above we can conclude that is significant to analyze three points and firstly the level of legislation, then regulations which include requirements about the health status of bulls and last but not least the quality of bulls in relation to the recommendations and rules.

There are differences at legislative levels that determine health conditions of bulls in Serbia, Bosnia and Herzegovina and the EU (Germany). In Serbia and Bosnia and Herzegovina those are determined by underlaw documents, in Germany by law and the EU by directive.

Regarding the requirement for the health inspection the bulls in Serbia, Bosnia and Herzegovina and the EU, there are certain differences. For example, serological tests are performed on each breeding bull in Serbia twice a year (in the EU 1x year), especially tests for EBL, BVD and IBR/IPV. Interestingly, the examination of bull semen using PCR method in Serbia is conducted for three viral infections: BVD, Schmallenberg virus (every six months) and IBR/IPV (every three months). Legislation in Serbia might require too much testing which is not necessarily scientifically and economically justified. For instance, in the EU the BVD control of semen applies only on BVD seropositive bulls. From a scientific perspective, it is questionable, when or in which time period we can detect shedding of infection agents in semen. This remark applies to the compulsory testing
of semen for *brucellosis, Campylobacter fetus* spp. *venerealis, Trichomonas foetus*, IBR/IPV in Bosnia and Herzegovina, which is not mandatory in the EU. These examinations raise the price of semen production, and make it less competitive on the market.

Generally, having in mind differences in subsidiary legal acts which are applicable in Serbia in relation to the EU, we believe that their adaptation is necessary in order to protect the health of breeding animals, and prevent the appearance of diseases in cattle. Consequently, we can increase the level of health status of breeding animals and fulfill the conditions for export of bull semen to all countries which are expecting the EU exporting certificates as well.

As well as to the health status of bulls, there are many legal restrictions on the semen production and trade in the Western Balkan countries in relation to Germany. We describe a detailed list of conditions regarding semen quality, while in Germany there are only some recommendations by the ADR (2006). These recommendations for native sperm are similar to those in Serbia (e.g. volume, sperm concentration, abnormal morphology and progressive motility), but for frozen semen are not so detailed requirements as to market in Serbia and Bosnia and Herzegovina. In any case (native or frozen semen), the recommendation of ADR (2006) does not contain a threshold for amount of bacteria, while in Serbia exist a maximal total number of bacteria, from 500 to 5000 CFU/ml.

It is interesting a comparison of regulations on semen quality. There can be partially comparable regulations on the quality of semen in Serbia and ADR recommendations (2006). Regulations in Serbia explain in detail the requirements on the quality of semen. We are of the opinion that this is a good solution, but only a certain period of time for adjustment to the EU free market. Unlike to Germany, in Serbia there is no association of cattle breeders. So there is no organization that wrote the recommendation on the quality of semen. On the other side, the recommendations of the ADR (2006) mainly deal with certain forms of bull infertility. This is understandable because of the fact that in Germany exist a number of semen collection centre and high turnover and trade of breeding bulls. While in Serbia, there are three centers (Velika Plana, Knjaca and Temerin) and only one in Bosnia and Herzegovina (Banja Luka in Republic of Srpska).

However, there are significant differences between regulations in Germany, Serbia and Bosnia and Herzegovina. Nevertheless, regulations related to semen quality ought not to be altered until the Serbian entrance to the EU. We believe that there would exist certain confusions among clients having the present market conditions in mind in case of allowing free distribution without any quality control.

Our opinion is that we should not allow free circulation of bull semen on the market at once, to be more precise, buying and selling of semen without any quality control. Because the "copy-paste" implementation of these EU rules would lead to a chaotic situation on the Serbian market. This applies particularly to many clients (farmers) who do not fully understand the term "quality of the semen". More precisely, they don't know how important this factor is in the conception of cows. Having in mind, that the client only buys semen which he considers of appropriate and satisfactory quality. This means to contain the required number of progressively motile spermatozoa in a dosage, respectively. The experience has shown, on the free market of semen, it is not always the case.

On balance, it should be outlined that there is one national laboratory which is officially accredited in Germany, whereas this is not the case in Serbia and Bosnia and
Herzegovina. After all mentioned, we could recommend good experience according to bull health control from Germany, and apply this model for the future use in Serbia. If we take as a basis the orientation of Serbia and other countries in the region accession to the EU, and the commitment to harmonization of regulations related to animal health there is space for correction and supplement of legislation relating to bull semen production and trade (especially export to “third countries” i.e. custom union).

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