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Effect of semen collection by transrectal massage of accessory sexual glands or artificial vagina on the outcome of breeding soundness examinations of Italian yearling beef bulls

Lakamy Sylla [a], Claudio Palombi [a], Giuseppe Stradaioli [b,*], Antonio Vagniluca [c], Maurizio Monaci [a]

[a] Department of Veterinary Medicine, University of Perugia, Perugia, Italy
[b] Department of Agricultural and Environmental Sciences, University of Udine, Udine, Italy
[c] National Association of Italian Beef Cattle Breeders (ANABIC), Perugia, Italy

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ABSTRACT

Although semen quality is one of the major traits that influence breeding soundness examination outcomes in bulls, field conditions occasionally do not allow for the collection of semen samples by means of an artificial vagina. The aims of the present study were to report the results of a large number of semen collections that were performed *via* the transrectal massage (TRM) of the accessory sexual glands of Italian yearling beef bulls and compare this semen collection method to the artificial vagina (AV) method in term of breeding soundness examination outcomes; furthermore, we determined whether the breed affected the semen characteristics. In the TRM group ($n = 475$), the semen samples were collected *via* TRM of the accessory sexual glands, and in the AV group ($n = 502$), the AV method was used. In the TRM group, semen samples were obtained from 81.3% of the bulls and penile protrusion was observed in 87.6% of the animals during semen collection. The sperm concentrations (920.5 ± 439.0 vs. $281.0 \pm 259.8 \times 10^6$/mL) and the percentages of total abnormal spermatozoa (22.8 ± 15.0 vs. 18.8 ± 12.9) were significantly higher in the AV group than those in the TRM group. The percentage of bulls that did not meet the minimum requirement for normal cells (≥70%) was 6.2% higher in the AV group than that in the TRM group ($P < 0.05$). Moreover, the samples collected from Chianina bulls by TRM exhibited a lower percentage of motile sperm and a higher percentage of abnormal spermatozoa when compared with the other two breeds. The major drawbacks of the TRM technique were the inability to conduct complete evaluation of the libido and mating ability of the yearling bulls, a significant reduction of the number of spermatozoa collected, and an increase in the variability of the semen characteristics due to breed. In conclusion, despite the drawbacks, TRM guarantees that semen evaluation can be conducted in cases in which the semen samples cannot be collected with the AV method.

1. Introduction

The genetic selection scheme adopted by the National Association of Italian Beef Cattle Breeders (ANABIC) involves the use of yearling beef bulls that are selected on the basis of productive performance tests with the purpose of

* Corresponding author. Tel.: +39 (0) 432558580; fax: +39 (0) 432558603.
E-mail address: giuseppe.stradaioli@uniud.it (G. Stradaioli).

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accelerating genetic selection for meat production. However, the use of young bulls as sires is associated with the risk of selecting animals that produce unsatisfactory ejaculates in term of the number and/or quality of the spermatozoa. Indeed, the early identification of bulls with good reproductive characteristics, such as desirable scrotal circumference (SC), sperm motility and morphology, will be of primary importance for breeding because it increases the fertility of the herds and improves the productivity of the progeny [1,2].

At present, the best way to evaluate the breeding potential of bulls is to perform a bulls breeding soundness examination (BBSE), which also excludes animals with either congenital or acquired abnormalities that can adversely affect breeding efficiency [3]. The BBSE guidelines were established by various veterinary organizations and consist of evaluations of SC, semen quality, physical soundness, and the general health conditions of the bull [4–6]. However, semen collection and analysis represents one of the most important steps in the prediction of the breeding potential of bulls, and the guidelines of the Society for Theriogenology (SFT) establish minimum recommended values for both sperm motility (30%) and morphology (70%) [4]. Although no predictions regarding the degree of fertility are possible based only on semen evaluation, it is possible to provide reliable indicators of future performance on the basis of animal's seminal characteristics. Several techniques are used to collect bull semen; the most commonly used techniques are the artificial vagina (AV), electroejaculation (EE), and transrectal massage (TRM) of the accessory sexual glands. The use of an AV is the least stressful and most physiological method of collecting semen. However, this method is considered to be too difficult, time consuming, and sometimes dangerous for routine semen collection from untrained and potentially fractious beef bulls that are not accustomed to the procedure [7]. Consequently, the semen from yearling beef sires is frequently not evaluated before the bulls are used for breeding purposes. To avoid this limitation, EE is typically used in many countries to conduct BBSEs, but this technique is prohibited or discouraged in several European countries [8,9]. Semen collection by TRM has been described in cattle for many years [10–13]. Using this method, it is possible to obtain ejaculates from untrained bulls without using any expensive equipment, and most bulls do not vigorously object to it [14]. This procedure has recently been the object of research that has confirmed its feasibility in both yearling and range beef bulls of mixed breeds and ages [7,8,15,16]. However, not all bulls allow the collection of semen, and the quantity and quality of semen are lower for those of samples collected by EE or caudal epididymal dissection [8,16]. Moreover, a trial of a small number of bulls was conducted to determine whether the semen samples collected by TRM are comparable with those collected by AV [17].

In a previous study that reported the results of BBSEs performed on yearling Italian beef bulls at the end of the feed-based performance test, we observed that semen cannot be collected from several bulls using the AV method [18]. This problem occurs because the time interval between the end of the performance test trials and the beginning of the auction sales of the animals is too short to complete the training of the shyer and fractious subjects. Therefore, we have decided to introduce the TRM method into the protocol of semen collection to solve this problem and test the qualities of semen from bulls that cannot be collected by AV. In the present study, we report the results of 11 years of semen analysis of ejaculates collected with either the TRM or AV methods and show that the breed of the bull affected BBSE outcomes.

2. Materials and methods

2.1. Animals and husbandry

The data gathered in this retrospective study correspond with semen collections and evaluations conducted at the Research Station of the ANABIC located in Umbria, which is a temperate climate region in central Italy (43° 02′ 30″ N, 12° 20′ 30″ E), from 2002 to 2013. A total of 977 6-month-old calves were used; these calves were negative for brucellosis, tuberculosis, bovine leukemia virus, infectious bovine rhinotracheitis, and bovine viral diarrhea virus when they were admitted to the testing station. The selection of the calves was based on the conformation traits, the sires' genetic indices, and cytogenetical screenings that were performed before the subjects were admitted to the 180-day growth performance test. Bulls of pooled breeds were maintained in groups of 15 animals in semioutdoor pens; they had *ad libitum* access to hay in addition to a concentrate ration. Each year, four groups of calves ranging in age from 4 to 6 months were created, and they completed the growth performance tests in March, June, September, and December. At the end of the growth performance test when BBSE test was applied, the bulls were uniform in age (13 ± 1 month) and body weight (558.3 ± 45.4 kg).

The study was approved by the University of Perugia's committee on animal care.

2.2. Artificial vagina group

Samples from 502 of the 977 bulls that were subjected to semen collection with the AV method were successfully collected. The distribution of bulls through the experiment is reported in Figure 1.

The animals were subjected to the BBSE as outlined by the SFT in 1993, and two ejaculates were collected 15 minutes apart from the bulls that were subjected to the AV method on a mount bull restrained in a short-sided breeding chute [4]. On collection, the first and second ejaculates were pooled, and the volume of each sample was recorded in a graduated test tube, the sperm motility was assessed using a phase contrast microscope that was equipped with a heated stage at × 200 magnification (TMS; Nikon, Japan), and the sperm concentrations were measured with a Bürker hemocytometer. To increase the repeatability of the evaluations of the spermatozoal progressive motility, the semen samples were diluted in saline, and 15-μL drops were placed on a preheated microscope slide and covered with a 20 × 20-mm coverslip. The morphologic features of the sperm were assessed by the