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Review article

Andrology laboratory review: Evaluation of sperm concentration

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ABSTRACT

This article is the result of the work of the andrology task-force of the Association of Applied Animal Andrology, American College of Theriogenologists, European College of Animal Reproduction, Society for Theriogenology, and National Association of Animal Breeders. It is intended to serve as a comprehensive reference on methods to evaluate sperm concentration and to contribute to the adoption of best practices in veterinary andrology laboratories. The information covered in the article includes sample preparation and the use of manual counts, spectrophotometers, computer-assisted semen analysis, NucleoCounter, and flow cytometry. Emphasis is given to the principles of the methods and equipment, performing the evaluation, and common mistakes and/or pitfalls. In addition, the precision and accuracy of the different methods are also discussed.

1. Introduction

Evaluation of sperm concentration is an essential component of semen analysis, and results are used, among others, for breeding soundness certification, diagnosis and/or prognosis of reproductive disorders, processing insemination doses, characterization of semen samples for trade, and assessment of treatment effects on sperm production (e.g., toxicology and nutrition studies). Despite these very significant implications, evaluation of sperm concentration is sometimes viewed as a trivial test and results are taken for granted without proper validation. A different reality exists however, as demonstrated by several multi-center studies involving human andrology laboratories. Reported interlaboratory coefficients of variation for sperm concentration results range from 23% to 73% [1], 53% to 80% [2], and 21% to 34% [3] for individual samples, underscoring the difficulty to compare results among laboratories and to generalize the findings of scientific studies.

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Although similar studies have not been reported in the veterinary literature, the predicament of animal andrology laboratories is likely not very different.

Discussions among members of the Association of Applied Animal Andrology and American College of Theriogenologists lead to formation of a task-force to address the lack of standard methods and quality control measures, and the almost nonexistence of formal training materials for veterinary andrology laboratories. In addition to members from the Association of Applied Animal Andrology and American College of Theriogenologists, members of the task-force were also appointed by the European College of Animal Reproduction, Society for Theriogenology, and National Association of Animal Breeders. Appointed specialists of the task-force bring together diverse experiences in academia, industry, and private practice in several domestic species. The present article is the first result of the work of the task-force and is intended to serve as a comprehensive reference on methods to evaluate sperm concentration and to contribute to the adoption of best practices in veterinary andrology laboratories.

2. Sample preparation

2.1. Species-specific considerations

Differences in the reproductive biology, including testicular size, sperm production capacity per testicular mass, epididymal sperm storage capacity, and ejaculate volume dictate the physiological differences in sperm concentration observed in the ejaculate among species. Different methods of semen collection, sexual stimulation, and the environment can also affect quantitative ejaculate parameters. In addition, the ejaculate of some species consists of distinct fractions that differ in number of sperm and also in other physical characteristics that might affect sperm concentration evaluation, such as viscosity, opacity, and presence of particles. In the latter case, the sample might have to be processed before sperm concentration can be accurately determined (e.g., removing the gel from boar and stallion semen). Because no practical existing method allows all sperm in a semen sample to be counted, a subsample is counted to make inferences on that of the whole sample. A critical objective is to obtain a representative sample that contains a sufficient number of sperm so that counts can be performed efficiently; the recommended optimal number of sperm to be counted varies according to the counting method. Therefore, the technician must take into account the method to be used and the expected sperm concentration in the sample to dilute the sample appropriately before evaluation. Dilution rates can range from 1:1000 for highly concentrated samples (e.g., ram semen) to 1:5 for less concentrated samples (e.g., boar semen).

2.2. Diluents

The basic property required of any diluent used for sperm concentration evaluation is the ability to disperse sperm and not interfere with the counting method. Therefore, diluents are usually translucent solutions that prevent sperm from agglutinating. Simple salt solutions (e.g., sodium chloride or sodium citrate), buffered solutions (e.g., sodium bicarbonate or phosphate), more complex media (e.g., TALP and HEPES), semen extender, and even distilled water can be used as diluents for sperm concentration evaluation depending on the counting method. Other required properties are specific to the counting method and/or application and might include sperm immobilization, disruption of the plasma membrane, and prevention of autofluorescence. Immobilization of sperm is essential when performing manual counts and might also increase the precision and accuracy of computer-assisted semen analysis (CASA) results [4]. Distilled water or 10% saline solution can be used to immobilize sperm because it results in osmotic shock. Dilution of bovine semen in diluent containing 30-mM sodium fluoride immobilizes sperm in a characteristically rigid form [5]. Sperm can also be immobilized by adding 0.35% formalin to the solution [6,7], but the solution must be tested before use because formalin may cause sperm agglutination when combined with certain salts and/or buffers. The NucleoCounter requires the use of a nonpermeable dye to stain sperm for evaluation of concentration. Therefore, a special diluent containing detergent is used to disrupt the plasma membrane and allow penetration of the dye into all sperm in the sample [8]. It has also been demonstrated that different media have different effects on sperm autofluorescence; therefore, these should be evaluated when employing methods that rely on detection of fluorescence such as flow cytometry [9].

2.3. Preparing dilutions

Because semen samples almost invariably have to be diluted before evaluation, obtaining reliable results require very accurate dilution. Because diluent and semen sample volumes are usually small and dilution ratios are relatively large, even minor sampling errors can significantly affect the results. Proper user, maintenance, and calibration of instruments used to prepare dilutions are essential.

Manual micropipettes are the most common instrument used for sampling and diluting semen for concentration analysis. Like all precision instruments, pipettes produce more reproducible results when operated with attention to detail and proper technique. Unfortunately, training on proper pipetting technique is often neglected, and calibration of instruments and evaluation of technician performance are afterthoughts at best. To ensure consistency, andrology laboratories should adopt standard operating procedures for pipetting techniques and ensure that all instruments and operators are periodically evaluated. Pipettes are classified as “air” or “positive displacement” according to the mode of operation. Air displacement pipettes have a piston in a cylinder that moves to the appropriate position once the volume is set. The volume of liquid aspirated or expelled is the same as the volume of air contained in the cylinder. Positive displacement pipettes also have a piston in a cylinder or capillary tube that moves to the appropriate position once the volume is set. However, the piston is in direct contact