

Modern Techniques for Semen Evaluation



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KEYWORDS

• Semen • Semen evaluation • Fertility • Stallion • Breeding

KEY POINTS

- The semen evaluation should be performed as part of a complete breeding soundness evaluation.
- The effect of mare and management factors should be taken into account when the results of the semen evaluation are interpreted.
- Understand how to use the tests and the limitations of each test.
- Test limitations can have a profound effect on the interpretation of the results.
- Test results should be interpreted accounting for the influence nonstallion factors may have on the presenting complaint (eg, subfertility).

INTRODUCTION

The evaluation of stallion semen is an integral part of evaluating horse subfertility and determining a stallions potential as a breeding prospect. Although semen evaluation is an important part of a stallion's breeding potential, good sperm quality is necessary, but not sufficient, for fertility. This concept is important because a semen evaluation is often performed as a separate "stand-alone test" with little knowledge of the limitations that may be imposed by the mare or management. In general, there are 3 factors that affect fertility: the stallion, the mare, and management (ie, human component). Often when the clinician is presented with inadequate fertility the stallion or the mare are assumed to be the source of the problem. Currently, there are many semen tests, most of which are sperm quality tests. Coincidental with the application of a sperm test the clinician should interpret the results of the test to the client. In general, sperm quality tests tend to be correlated with other sperm quality tests (ie, if one is "good" others are also "good") and oftentimes the clinician and client may interpret additional testing as providing little value. However, these tests can provide unique information about individual stallion that is not obvious from results of other tests.

In general, there are 2 circumstances when a semen evaluation is performed (1) as a stand-alone test when the fertility of the stallion is in question owing to poor fertility

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outcome and (2) to determine a stallions potential fertility stallions may or may not have a prior breeding history.

In all species, there is a constant pursuit of the holy grail of sperm quality tests. Essentially, a single test, that when applied to a semen sample, explains/predicts stallion fertility. This is a naïve pursuit; as veterinary clinicians, we understand implicitly that horse fertility is composed of much more than sperm quality. In addition, there are many self-imposed limitations to how the evaluation of sperm quality is performed as well as what tests are used and how they are interpreted. Understanding these limitations is critical as the clinician is required not just to perform the test but to interpret the results.

Although there have been many new “sperm tests” introduced relatively recently, few are validated regarding their relationship to fertility. The clinician should be cautious in promoting tests unless interpretation of the results relative to fertility can be presented to the client.

SPERM MOTILITY

Sperm motility is, historically, the most common test of sperm quality, primarily because of the ease of evaluation under a variety of conditions. Biologically, the ability of a sperm to be motile is certainly necessary for fertility, but the type and quality of motility can be affected by the conditions (eg, hot, cold, osmotic changes) under which the test is determined. Therefore, the sperm motility test should be conducted under well-controlled conditions, such that the results reflect the inherent sperm quality of the stallion tested and not iatrogenic influences. If these conditions cannot be accommodated, then the results should be interpreted accordingly. Ideally, sperm motility should be tested using a phase-contrast microscope with both $\times 20$ and $\times 40$ objectives such that the final magnification should be $\times 200$ and $\times 400$, respectively. The microscope should be equipped with a warm stage that can be adjusted to 37°C . These magnification levels allow a clear visualization of a low- and high-power view of the sperm sample. Light microscopy should be avoided because clear visualization (ie, the ability of the eye to identify immotile sperm), especially at a low magnification, may be insufficient. The inability to identify immotile sperm may result in a falsely high motility value.

Microscopically, sperm motility is evaluated under a variety of conditions, all of which can result in a value that may inaccurately reflect stallions inherent sperm quality. The magnification (eg, $\times 100$ – $\times 1000$), type of microscope (eg, light, phase contrast), working conditions (eg, heated stage, ambient temperature), evaluator experience, as well as unknown influences that may be toxic to sperm, are just a few examples of factors unrelated to the intrinsic sperm motility of the stallion. Therefore, the clinician should be cautious about the interpretation of sperm motility results when they seem to be inconsistent with other clinical findings.

Adding to the subjectivity of evaluating sperm motility are the different types of sperm motility evaluated, which include total motility (TMOT) and progressive motility (PMOT). Of these 2, PMOT is the most problematic. Sperm that are “progressive” are considered to be moving in a relatively straight forward motion and are assumed to be more “fertile.” Historically, sperm progressivity is assumed to imply normality, whereas other nonprogressive sperm are considered pathologic. As the evaluation of sperm quality has evolved and additional sperm quality tests have been introduced, it has become clear that the measurement of progressive sperm motility is outdated. The factors mentioned that lead to variation are particularly worrisome when PMOT is evaluated. These artifactual factors have the potential to render a low PMOT value in a

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