Prebreeding season/prepurchase examination of the stallion: testicular ultrasonography, semen evaluation, and considerations

Camilo Hernández-Avilés, Charles Love
Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences
Texas A&M University, College Station, TX

Abstract

The stallion breeding soundness examination (BSE), as initially contemplated by the Society for Theriogenology, was intended to serve as a tool for selecting stallions that under good breeding management and mare fertility, were expected to efficiently render at least 75% of > 40 mares pregnant when bred naturally or 120 mares when bred artificially in 1 breeding season. Since its initial introduction 38 years ago, major changes in the equine breeding industry have occurred. Yet, the original purpose of the BSE has remained somehow unchanged and a BSE still provides to both practitioners and owners valuable information regarding the reproductive potential of the stallion and reproductive management options that could be implemented for optimizing fertility of a particular stallion. This manuscript discusses some aspects of BSE that warrant special considerations, due to their implementation under field conditions and their interpretation regarding the potential fertility of stallions.

Keywords: Stallion, breeding soundness, testes, semen quality, fertility

Introduction

In 2021, the American College of Theriogenologists celebrates its 50th anniversary. Such an historical occasion warrants a very succinct, but necessary, reminder of the original purpose that led to the creation of an organization dedicated to the study of animal reproduction, Society for Theriogenology (SFT), and the veterinary specialty that today we celebrate, Theriogenology. During its early days, the SFT established a series of guidelines for determining the reproductive potential of bulls. Such guidelines eventually became what is today known as the Society for Theriogenology manual for breeding soundness examination (BSE) of bulls, initially published in 1983, reviewed in 1992, and revised in 2018.[1] Given the increasing interest of veterinarians, reproductive physiologists, and owners in the development of a standardized method for selecting and predicting the potential fertility of males in food and companion animals, similar guidelines were also established for boars (1984),[2] small ruminants (1980),[3] dogs (1992),[4] and stallions (1983).[5] Despite some differences in the breeding management and expectations amongst various breeding industries, most of the guidelines for the male BSE follow a similar approach. These include the analysis of general health status, evaluation of the reproductive tract, libido and mating ability assessment, semen analysis, evaluation for venereally transmitted diseases (including those that are reportable), and analysis of breeding records (when available).

The stallion BSE was a product of a committee led by prominent theriogenologists and equine practitioners, including Robert Kenney and John Hurtgen (University of Pennsylvania), Robert Pierson (Colorado State University), Don Witherspoon (Lexington, Kentucky), and John Simons (Lexington, Kentucky). This Manual also incorporated comments from worldwide leaders in the study of equine reproduction at the time, including John Hughes (University of California at Davis), Atwood Asbury (University of Florida), Wendell Cooper (University of Pennsylvania), Hans Merkt (University of Veterinary Medicine at Hannover, Germany), Marcel Vandeplassche (Ghent University, Belgium), Wladyslaw Bielanski and Marian Tischner (Krakow Agriculture University, Poland), among others. The list of individuals who in major or minor extents contributed to what is nowadays the stallion BSE leads us to appreciate how this manual attempted to be internationally inclusive, scientifically based, and applicable for field situations by theriogenologists and general equine practitioners.

From a comparative standpoint, it is worthwhile to remind practitioners and trainees that bulls, rams, bucks, and boars are mostly selected based on reproductive performance. Other aspects related to genetic merit or physical conformation are only taken into consideration after a male has been proven to be potentially fertile. Likewise, the results of a BSE conducted in a food animal will assist in identifying males that do not have certain physical, behavioral, and reproductive characteristics above a previously established cut-off value. Such individuals will be eliminated from the breeding population. In contrast, the genetic merit or pedigree, sports performance, or conformation will be always a priority when selecting a stallion for breeding purposes. The main goal of the stallion BSE will be, thus, to identify the cause(s) of potential reduced fertility, and pursue, when possible, an alternative for the breeding management. Exceptions to this will be cases of stallions with hereditary conditions (i.e. HERDA, HYPP), or physical abnormalities that will largely hamper their breeding performance (i.e. Wobbler Syndrome, neurologic disease). As such, it will be very uncommon to cull stallions from a breeding farm due to results of a BSE.

Salient points of stallion BSE

Although the main objective of the present document is not to review thoroughly what is or what is not included in the original manual for the stallion BSE,5 it is important to ponder certain concepts mentioned in that document that might warrant special attention by the audience. Some of these include:

1. **'The examination (BSE) will assist in identifying the cause(s) of reduced fertility and the findings used to develop guidelines for the management of the stallion to enable it to achieve its maximum fertility'.** A stallion that does not 'pass' a BSE can still be fertile if adequate alternatives for breeding management are done. Some stallions perhaps will never 'pass' a BSE, and yet, can be utilized as studs with relative success.

2. **'It is realized that this examination may not invariably and reliably predict the level of fertility that any particular stallion will achieve under a multitude of management conditions'.** Assuming that the BSE will confidently predict the reproductive potential of a stallion is erroneous. This is particularly true when the breeding management and intrinsic fertility of the mares bred to a stallion are not taken into consideration.

3. **'Fertility predictions are made on the basis of the findings at the time of examination. We realize intrinsic fertility may vary over a period of time'.** The BSE will only determine the reproductive potential of a stallion for a specific time: the day of examination. It cannot be expected that the results of a BSE will predict the performance of a stallion in the years to come, not even in the same breeding season in which the stallion is evaluated.

4. **'There is no single physical or seminal parameter which is satisfactorily correlated with the fertility of the stallion and the best combination of measures remains to be determined'.** Practitioners, scientists, and breeders have always attempted to find a single test that can reliably predict male fertility. The only test that might fulfill this purpose is breeding fertile females under good management conditions. Obviously, in vivo fertility trials are not an option when determining fertility potential of stallions. As such, the BSE should always take a holistic approach. This implies the inclusion of multiple sperm quality assays that are logical and have scientific merit. Also, interpretation of these assays should not be only based on high or low values compared to an arbitrary cut-off. Rather, their interpretation should take into consideration the potential causes of a 'low' or unusual value and their implications to the breeding method and management used for that particular stallion.

5. **'Fertility evaluation of stallions is not a precise science… The major variables affecting the results are the innate fertility of the band of mares the stallion is bred to, the overall management of both the stallion and the mares, the veterinary management, and last, but not least, the quality of the performance of the tests as well as the quality of the interpretation of all findings'.** This last sentence summarizes the 4 aspects that were discussed above. Of interest is the phrase regarding the quality of tests and the interpretation of all findings during BSE. Practitioners must be aware that any sperm quality test is prone to errors, due to the nature of the test, errors when performing such evaluations, or the limitations and competence of the evaluator. The concept 'garbage in – garbage out' is very relevant in this situation.

Impact of new techniques for equine breeding management on stallion BSE

At this point, it is also worthwhile to recall the suggestions in the stallion BSE manual regarding the expected levels of fertility that should be achieved by potentially satisfactory breeders. A stallion would be classified as 'satisfactory' if his physical, behavioral, and reproductive characteristics would allow him to render pregnant at least 75% of 40 mares (30/40 mares) bred by natural breeding, or 120 mares (90/120 mares) bred by artificial insemination. In modern equine breeding industry, it is not uncommon to find very popular stallions to be booked to 100 - 200 mares in the Thoroughbred or > 200 mares where artificial insemination is allowed (i.e. Quarter Horses and Warmbloods). Additionally, advances in semen preservation technologies and artificial insemination, including cooled storage, freezing/thawing, low-dose insemination, estrus, and ovulation synchronization, and more recently intracytoplasmic sperm injection (ICSI), have allowed high fertility to be achieved in selected individuals that otherwise would not be considered as potentially fertile, based on the guidelines established initially by the manual. The readers are referred to some previous works that have illustrated specific circumstances of breeding management for the subfertile stallion to achieve optimal fertility.[6-9]

That many stallions have benefited from the use of modern methods for semen processing, storage, and insemination does not imply that the manual for the stallion BSE is outdated nor useless. This manual presents a very effective and thorough framework to investigate obvious causes of potential subfertility in stallions, and can be also used as a starting point to monitor the reproductive performance of stallions that just began their reproductive career. Hence, theriogenologists, trainees, and general practitioners are encouraged to adopt the BSE as the method of choice for the estimation of reproductive potential in stallions, either when presented the first time for stud, before every breeding season, after the occurrence of any health problem that might impact his reproductive potential, or as part of a prepurchase examination.

Testicular evaluation in the context of BSE

Evaluation of scrotal contents in the stallion has received special attention by practitioners and owners, particularly in the case of the Thoroughbred. In this industry, when a stallion is retired to stud, it is common by the 2 parties involved (buyer and seller), to sign a policy for first-season subfertility insurance. If complaints regarding the breeding performance of the stallion appear while in his first season as a stud, a veterinarian is required to conduct an exam on behalf of the policy underwriter. Most of these examinations are based on assessment of the stallion's general health status and evaluation of scrotal contents, particularly testicular size. Unfortunately, veterinarians are asked to render an opinion about the potential fertility of the stallion in question, without the option of examining other aspects of reproductive performance, including libido, mating ability, ejaculatory function, or semen quality. The results of the first-season subfertility insurance are used to judge if the stallion is likely to achieve a minimum seasonal pregnancy rate of 60% when booked to a defined number of mares. In addition, it is also expected that the veterinarian recommends the maximum number of covers per day (or per week) that should be allowed for that stallion.[10]

Stallion testicular size can be determined by measuring either total scrotal width (TSW, cm) or total testicular volume (TTV, cm^3). Total scrotal width, is the largest measurement taken directly across both testes and the scrotum, commonly done with calipers. Light breed stallions had a mean (± SD) of 10 ± 0.9 cm TSW when measured during the physiological breeding season (April - September).[11] A difference in TSW was noticed for stallions ≥ 7 years old when compared to stallions ≤ 6 years old (10.9 cm versus 9.6 -10 cm, respectively; $p < 0.05$). Similar values (10.1 - 10.8 cm TSW) were reported for Standardbred stallions, located either at the racetrack or the breeding farm.[12] In that study, younger stallions that also were actively trained or were exposed to medications while at the racetrack had smaller TSW than nonmedicated or breeding stallions. Values for TSW for Thoroughbred or Standardbred stallions (n = 106, 3 - 6 years) recently retired from racing were 9.2 - 10.3 cm.[13] These workers indicated that 20% of stallions that did not fulfill the criteria to be considered as a 'satisfactory prospective breeder' also had a TSW < 8 cm. It is commonly assumed a TSW value > 8 cm as 'normal' for breeding stallions.[14] Yet, to date, studies in which a direct relationship between TSW and stallion fertility are lacking, and probably it would not be possible to establish such a relationship, given the complex nature of stallion fertility and breeding management.

Determination of total testicular volume (TTV, cm^3) is performed either by estimating testicular measurements using a caliper or ultrasonography (Figure 1). For both techniques, the stallion should be placed in a palpation stock and sedated with the aid of α2-agonists given intravenously (e.g. xylazine hydrochloride: 1.1 mg/kg or detomidine hydrochloride, 20 - 40 µg/kg). This helps in the descent of both testes into the scrotal sac, facilitating reliable measurement. Regardless of the method used, the practitioner must estimate 3 parameters (length – L, width – W, and height – H) for each testis. Given that the shape of the stallion testis resembles an ellipsoid, testicular volume can be confidently calculated by using a formula to estimate the volume of an ellipsoid: $4/3\ \pi \times L/2 \times W/2 \times H/2$ or $0.5236 \times L \times W \times H$, inputting values in cm from each

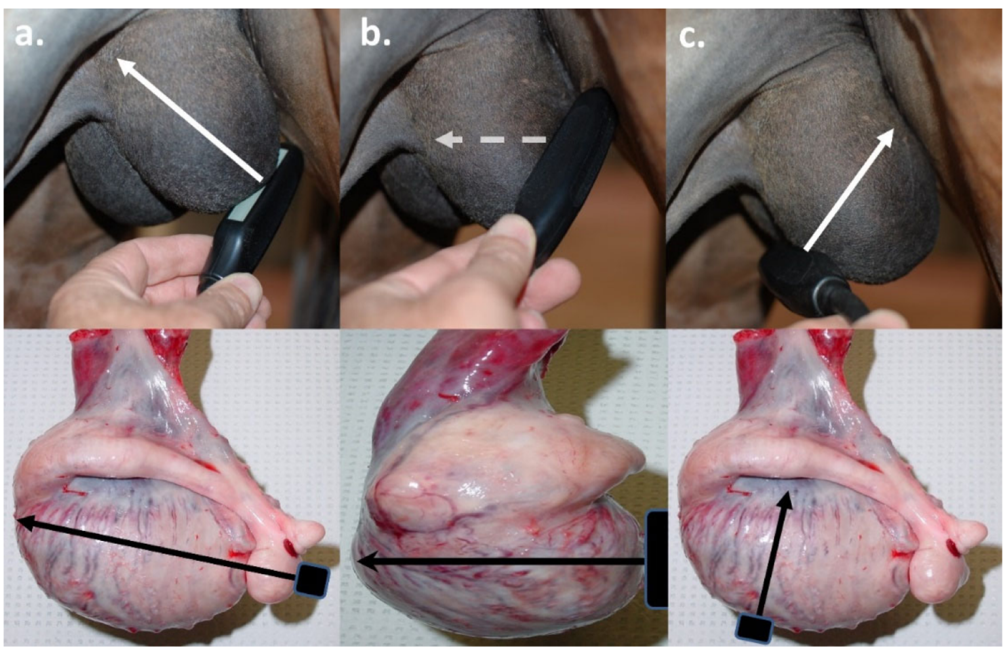

Figure 1. Estimation of total testicular volume (TTV, cm^3) by measuring 3 dimensions of each testis: a. length; b. width; c. height. Images taken using an anatomical specimen are also shown for proper orientation of the ultrasound beam.

testicular measurement.[15] Results obtained from each testis are added, and the combined testicular volume is also expressed as the combined testicular weight in grams (g). Similar results of TTV can be obtained when using either caliper or ultrasound measures.[15] Yet, the use of ultrasonography for determining TTV might be advantageous, given that more precise measurements of the testicular parenchyma are obtained, without the interference of scrotal skin or other abnormalities such as hydroceles that can artifactually increase TTV. Substantial inter-operator variability can be expected when using either technique; therefore, readers are referred to a method used by the authors to properly determine TTV with ultrasonography.[16]

The relationship between TSW or TTV and daily sperm output (DSO) has been established.[15,17,18] This is of great help for estimating the number of mares that can be bred daily, or the number of seminal doses that can be produced for artificial insemination using fresh, cooled, or frozen/thawed semen. TSW, as determined by caliper measurements, accounted for up 60% of the variation in daily sperm output of stallions whose semen was collected for 10 consecutive days.[17] TTV, as determined either by caliper or ultrasound measures, accounted for up to 85% of the variation in daily sperm output of stallions whose semen was collected for 7 consecutive days.[15] In that study, the formula that best established a relationship between TTV and DSO was (0.024 x TTV)-1.26 (Figure 2). An additional calculation is replacing 1.26 by 0.76.[18] This is to establish a range of DSO for the estimated TTV in that particular stallion. Based on previous studies,[15,18] mature stallions (4 - 6 years), including Thoroughbreds, Standardbreds, Warmblood, and Pony stallions, have an average TTV of 250 cm^3 Using the regression formulae mentioned above, it can be expected that such stallions produce 4.74 - 5.24 x 10^9 sperm if collected when at DSO. Under ideal conditions, the relationship between TTV and DSO should be performed in stallions that have been collected for several days, to deplete extragonadal sperm reserves and have a better appreciation of the amount of sperm that the testes are capable to produce on a daily basis.[17,19] However, attempting to collect a stallion daily for 7 - 10 days is impractical, due to monetary and time limitations. Even when the stallion DSO is affected by the testicular size, the earliest in which DSO is expected to be stabilized is after 3 - 5 consecutive days of semen collections in stallions with small testes (148 - 245 cc^3).[20] Hence, it is often easier to collect 2 ejaculates, 1 hour apart – as described in the stallion BSE manual – to have a crude approximation of the potential DSO for that particular stallion.

The application of TTV and predicting DSO is limited unless the efficiency of the testes to produce sperm is not considered. Some stallions are presented with small testes, yet high testicular efficiency, whereas other stallions can have larger but inefficient testes. Rather than comparing TTV and DSO against certain reported values, it is more convenient to determine if the amount of sperm ejaculated by that stallion corresponds to his predicted DSO. This concept, known as spermatogenic efficiency, is the product of *Actual DSO/Predicted DSO*, where actual DSO is the total sperm number obtained after collecting semen from the stallion at DSO. It becomes obvious that normal spermatogenic efficiency should be as close as possible to 100%. A dramatic decrease in spermatogenic efficiency can be a consequence of transient testicular dysfunction, or permanent testicular degeneration, depending on the chronicity of the insult to the testes. A marked reduction in spermatogenic efficiency is most common in aged stallions with declining testicular function; often this is the earliest indicator of testicular dysfunction despite no change in testicular size.[21] As a side note,

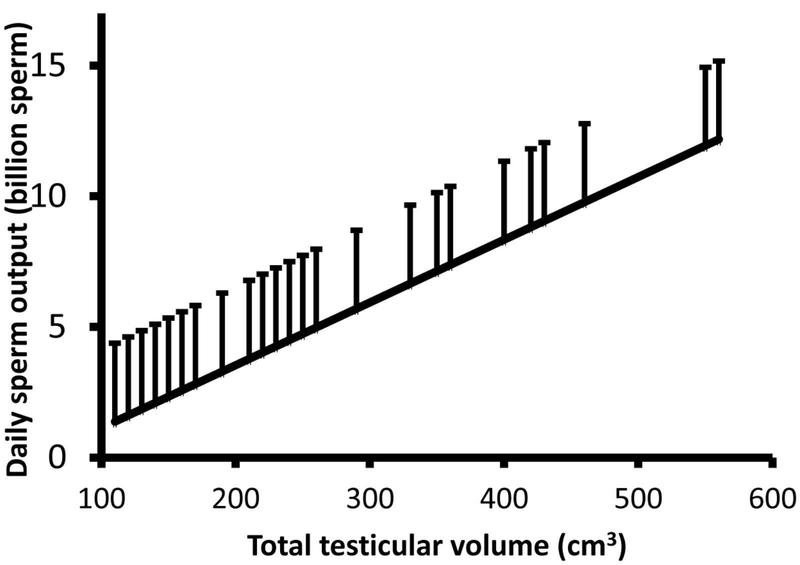

Figure 2. The linear relationship between total testicular volume (TTV, cm^3) and daily sperm output (DSO, 1 x 10^9 sperm), as determined by the formula (0.024 x TTV)-1.26. Adapted from Love et al.[15]

the spermatogenic efficiency per gram of testicular parenchyma has been reported in the stallion. For mature, sexually active stallions, it is estimated that 1 gram of functional testicular parenchyma can produce 14 - 18 x 10^6 sperm/day.[22,23] In aged stallions, values approaching 6 - 8 x 10^6/g/day are concurrent with a decrease in total sperm number in each ejaculate at DSO (< 2 x 10^9 sperm), and reduced pregnancy rates.[21] This suggests that spermatogenic efficiency and spermatogenic efficiency per gram of testis are useful indicators of general testicular function in the stallion, and their estimation should always be included when evaluating testicular size during the BSE.

Semen analysis and its potential relationship to stallion fertility

Perhaps, semen analysis and its interpretation has been considered as the main component of the stallion BSE. Although this assumption is not completely erroneous, judging the potential fertility of stallions based solely on the results of a spermiogram can lead to clinical errors. The document presented in this issue by authors discusses to a greater extent the methods that are commonly used for evaluation of semen quality in the stallion. Yet, it is worthwhile to briefly discuss some of these techniques in the present document.

Historically, semen analysis of the stallion BSE included estimation of total sperm numbers in the ejaculate, being the product of the gel-free seminal volume (ml) multiplied by the sperm concentration per ml (1 x 10^6), the estimation of both total and progressive sperm motility, and the evaluation of sperm morphology characteristics. These semen assays were considered to allow practitioners to conduct a BSE under field conditions, using only limited and inexpensive equipment. Also, the inclusion of these types of tests has its origins in what was previously done in other species, such as the bull, and what other equine researchers at the time had also observed.[24-26] Such analyses were mainly conducted using light, or phase-contrast microscopy for estimation of sperm motility and morphology, and direct enumeration methods for quantification of sperm numbers (e.g. hemocytometer). Although the stallion BSE manual recommended phase-contrast microscopy for assessing sperm motility, and particularly for sperm morphology, these authors also realized that using techniques compatible with a light microscope, such as stained smears, was more convenient for some field situations. Thus, most practitioners in the field prefer to use light microscopy-based techniques for semen analysis. However, under certain circumstances, such techniques might represent a potential limitation for objective analysis of stallion sperm quality, leading to misinterpretation and misjudgment of a stallion's potential fertility.

Based on the stallion BSE manual, a potentially fertile stallion should ejaculate at least 1 x 10^9 morphologically normal, progressively motile sperm in the second ejaculate each month of the year, after 1 week of sexual rest. Workers in The Netherlands, using a similar evaluation system to the stallion BSE Manual, reported a lower threshold for the number of morphologically normal, progressively motile sperm in the ejaculate (500 - 600 x 10^6) can be considered as acceptable for breeding stallions.[27] However, using such 'threshold values' is problematic, particularly in stallions with excellent sperm quality that require considerably fewer sperm to render fertile mares pregnant under adequate breeding management, or stallions that have acceptable semen quality and yet require to be bred to few mares for optimal fertility. This is particularly challenging when evaluating stallions for artificial insemination programs, given that certain minimum values for sperm numbers must be accommodated when breeding mares (a.k.a. 'breeding dose'). Even when some standards have been set for what is known as a seminal dose (e.g. fresh semen on the farm = 500 x 10^6 progressively motile sperm; cooled semen = 1,000 x 106 progressively motile sperm; frozen semen = 250 x 10^6 progressively motile sperm), these standards are merely based on industry expectations rather than reliable scientific data. For some stallions, these standards might represent an inefficient use of semen, due to their high intrinsic fertility, whereas for other stallions, greater numbers of sperm should be included in a 'dose' to achieve optimal fertility. Also, it is important to acknowledge that many breeding scenarios and management systems can confound the expectations of semen quality and its relationship with stallion fertility. A clear example of this is Thoroughbred stallions that have 'low' sperm quality but can still manage a relatively large book of mares with appropriate reproductive management of both the stallion and mares.[28]

A common question asked by practitioners and owners when conducting a stallion BSE is to what extent do these semen quality tests reliably predict fertility? If the relationship between the seminal traits of a particular stallion(s) and his (their) fertility is high (often estimated in research scenarios by product-moment correlation analyses), then it is assumed that the prediction capacity of the semen assays is good. An example of this would be a stallion with excellent semen quality that deposits sufficient numbers of 'good' sperm in the uterus of a fertile mare. A similar scenario will be that of a stallion with poor semen quality, who is bred to fertile mares and does not render them all pregnant. In both cases, the relationship between sperm quality and fertility will be linear (either positive or negative), leading to the assumption of a high predictive value of semen analysis over fertility. Nonetheless, many of the studies that had attempted to establish a relationship between stallion semen quality and fertility have not taken into consideration either intrinsic differences amongst stallions in sperm quality or potential differences in mare intrinsic fertility and breeding management for that particular stallion.[29,30] Hence, for some practitioners and scientists, the relation between semen quality and fertility appears nonexistent. To the authors' knowledge, few published studies have attempted to demonstrate the relationship between sperm quality traits and stallion fertility, when mare intrinsic fertility and breeding management are somehow controlled.[31-36] Of these, it is worth mentioning that stallions with high values of sperm motility (total, not necessarily progressive), viability (i.e. plasma membrane intactness), normal morphology, and DNA intactness (as determined by the sperm chromatin structure assay), tend to have higher fertility. Yet, in these studies, a combination of these assays, rather than the use of just 1, has allowed establishing a direct relationship between semen quality and fertility. This latter aspect emphasizes the concept of using a holistic approach whenever evaluating semen quality in a stallion as part of the BSE.

Another common question regarding semen analysis whilst conducting a BSE is the use of threshold values to describe the quality of an ejaculate, and thus, the potential fertility of a stallion. In other domestic species, threshold values have been established for percent progressive motility (e.g. > 30% for ruminants,[1] > 70% for boars,[2] > 70% for dogs[4]), and percent morphologically normal sperm (~ 70 - 75% for the species aforementioned). Although the stallion BSE Manual does not contemplate a minimum percent of morphologically normal nor progressively motile sperm, it is common to observe in many academic and nonacademic publications that such values are assumed to range from 60 - 70% for fertile stallions.[14, 24-27] We agree that the higher the percentage of morphologically normal sperm and motile (not necessarily progressively motile) sperm in the ejaculate, the higher will be the potential fertility of the stallion. It is common to observe stallions with relatively 'low' percentages of both progressively motile sperm, or morphologically normal sperm that also have adequate fertility. Such stallions might ejaculate enough sperm to compensate for the 'lack' of progressively motile or morphologically normal sperm. This is most common in Thoroughbred stallions, although similar results can occur in stallions used for artificial insemination. Data from 2 stallions with distinctinctly different fertility rates, despite 1 of them having somehow 'better' sperm quality, are shown in Table 1.

The sperm motility and viability in both stallions are similar and can be considered high. Sperm DNA integrity for both stallions were also similar to those in fertile stallions.[33] Two noticeable differences in terms of sperm quality can be observed: 1) Whereas the AQHA stallion had a lower testicular size, his

Table 1. Semen quality of a 6 year old AQHA stallion with excellent sperm quality and low reproductive efficiency (2 breeding seasons), and a 10 year old Thoroughbred stallion with acceptable sperm quality and high reproductive efficiency (5 breeding seasons)

Sperm quality parameter	Stallion A (6-year AQHA)	Stallion B (10-year TB)
Total testicular volume (cm3)	214	413
Total sperm numbers (at DSO; x 109)	3.9 - 4.4	3.6 - 5.7
Spermatogenic efficiency (%)	89	66
Sperm total motility (%)	80 - 90	73 - 78
Sperm progressive motility (%)	68 - 85	47 - 59
Morphologically normal sperm (%)	56 - 61	47 - 59
Abnormal heads (%)	6 - 18	10 - 13
Detached heads (%)	0 - 1	7 - 22
Proximal droplets (%)	12 - 26	58 - 67
Distal droplets (%)	3 - 7	4 - 11
Bent midpieces (%)	0 - 2	1 - 5
Bent tails (%)	0	2 - 3
$COMP_{\alpha\text{-}t}$ (%)	8 - 10	14 - 25
Mare book	41 - 47	54 - 166
Seasonal pregnancy rate (%)	47 - 49	77 - 91
Per cycle pregnancy rate (%)	27 - 28	59 - 69

DSO = Daily sperm output
$COMP_{\alpha\text{-}t}$ = Cells outside the main population (DNA-damaged sperm, SCSA)

testes were more efficient in producing sperm than those of the TB stallion. 2) The TB stallion had a lower percentage of morphologically normal sperm due to the presence of a considerable proportion of detached heads and proximal droplets. Why then did the AQHA stallion had such lower reproductive efficiency? Perhaps, the TB stallion ejaculated enough morphologically normal sperm into the mare's uterus to compensate for the presence of abnormal sperm. One might assume that only morphologically normal sperm can be both motile and viable, but based on research in our laboratory, sperm with either proximal or distal droplets are more likely to be viable than morphologically normal sperm (OR: 4.95; $p < 0.0001$).[37]A different scenario would have occurred if this TB stallion was used for a program of artificial insemination with cooled or frozen/thawed semen, since morphologic abnormalities observed can negatively impact the longevity of stored sperm. Also, even though the percent of morphologic abnormalities in the TB was considerably 'high,' this type of sperm abnormality perhaps did not interfere with his fertility, as neither proximal nor distal droplets negatively impact the likelihood of stallion sperm to establish a pregnancy when natural cover is used.[38] For the AQHA stallion, the picture is more complicated because this stallion has adequate-to-excellent sperm quality; thus, an explanation for his reduced fertility can not be obtained based on the BSE. One might argue that his testicular size could be a limitation for obtaining adequate fertility, but his testes were more efficient in producing sperm than in the TB stallion, and the AQHA stallion bred fewer mares than the TB. At this point, we speculate that the cause of reduced fertility in the AQHA stallion was due to breeding management, rather than to intrinsic sperm quality. In stallions with acceptable sperm quality, mare intrinsic fertility and breeding management are the main limiting factors for adequate fertility when artificial insemination with cooled semen is used.[39].

Evaluation of breeding records: the good, the bad, and the ugly

If evaluation of scrotal contents or semen quality in the stallion might present a certain degree of difficulty for some practitioners, the analysis of the reproductive history or breeding records can become the most challenging part of the BSE. One of the reasons is the lack of a consensus when defining reproductive efficiency. Many definitions of fertility can be equally true, depending on to whom the question is asked. For instance, the owner of the stallion will be interested in the number of foals that are born during the season after breeding his stallion to a group of mares. This definition of fertility is known as **foaling rate (FR)**, and in many equine breeding industries, is the most important measure of breeding efficiency, given its economic implications. Foaling rate is not a fair measure of stallion fertility, just because mares can become pregnant at an efficient rate due to the high intrinsic fertility of both the mare and the stallion, and foaling rates might be reduced due to unexpected events, such as abortion or perinatal losses. Another definition of fertility is the **seasonal pregnancy rate (SPR)**, being the number of mares that became pregnant after being bred to a stallion during the breeding season divided by the total number of mares bred to that stallion.[40] This parameter was historically considered the definitive measure of fertility and has been used in various studies that attempted to estimate the relationship between sperm quality and stallion fertility. Although it seems to be a logical parameter for the quantification of fertility, some stallions can have a high SPR despite being subfertile. These stallions can have similar SPR to fertile stallions, but be less efficient and require more breedings to achieve a high SPR. It is considered that under well-managed breeding systems, SPR should be at least 75%.

A better definition of intrinsic stallion fertility is the **per cycle pregnancy rate (PC-PR)**, which is the total number of mares pregnant at the end of the season divided by the total number of mare cycles bred.[40] The assumption is that all mares that are bred are of normal fertility and cyclicity and that breeding occurred when the mare was in standing heat and close to ovulation. In general terms, determining PC-PR allows eliminating certain confounding factors that can affect stallion fertility and might facilitate identification of highly fertile stallions that can render pregnant the mares of their book in fewer cycles, from those subfertile stallions that require multiple cycles. In general terms, PC-PR for commercial stallions approaches at least 50 ¬- 60%; therefore, on average, a stallion will require 2 cycles or less to render mares pregnant. Still, this parameter can be problematic when addressing causes of stallion subfertility, particularly in mares bred via artificial insemination, as they are often bred multiple times in the same cycle, or in mares bred either by live cover or artificial insemination due to the intrinsic fertility of the mares bred to that stallion. Hence, it would be advantageous to determine the **first-cycle pregnancy rate (FC-PR)**, that is the number of mares pregnant to the first breeding of the season, divided by the total number of mares pregnant that season.[40] By using this parameter, negative effects of inherently subfertile mares on stallion fertility can be minimized. However, this parameter is oftentimes hard to measure, particularly without adequate breeding records. Of this last parameter, caution must be exercised for mares that foal later in the breeding season, as they have a lower chance of getting bred and conceive on the first cycle, which in turn also would artifactually decrease FC-PR for that stallion.

A final consideration regarding the evaluation of breeding records is to determine the types of mares booked to a particular stallion, and expectations of the stallion's owner regarding his fertility. Breeding a fertile stallion to maiden or foaling mares is very common when the stallion is at his first(s) season(s) at stud. If the resulting foals are of high merit, then it is very likely that this stallion will be booked for subsequent seasons to mares of high fertility. Conversely, it is also common to observe highly fertile stallions to be bred to barren mares when the stallion's popularity decreases, which might reduce his fertility due to 'nonstallion factors,' as illustrated in Table 3. The mare class (maiden, barren, 'slipped', in-foal, not bred) that is bred to a

Table 3. Fertility results from a 22-year-old Thoroughbred stallion with a book of 27 mares

Class of mare	SPR	PC-PR
Barren	2/3 (67%)	1/7 (29%)
Foaling	12/22 (86%)	19/32 (59%)
Slipped	2/2 (100%)	2/4 (50%
All classes	23/27 (85%)	23/43 (53%)

stallion can reveal very important information regarding the intrinsic fertility of that sire. As such, practitioners should become familiarized with various definitions for mare class in the breeding context, and its impact on the potential fertility of a stallion. A more detailed description of this is provided.[40,41] Regarding the second aspect, theriogenologists and general practitioners often ignore what are the expectations of the owner regarding the potential fertility of a stallion. It is assumed that if a stallion can render pregnant at least 40 mares by natural cover or 120 mares by artificial insemination, then he can potentially have more mares booked to him. Yet, some owners (particularly in the artificial insemination industry) do not desire to breed so many mares. For some of them, having their mares bred to that stallion in question, or perhaps some mares locally are enough reward to their economical and time investment in the stallion. We have had instances in which owners present their stallions for a BSE, and their desire is not to breed more than 10 - 20 mares, mostly via 'live cover'. In those cases, such individuals will be exceptionally fertile if their breeding management is adequate. Eventually, some of these stallions are presented in subsequent breeding seasons for 'reevaluation' and either the owners decide to increase or decrease their books. Adjustments regarding the breeding management of these stallions are done accordingly.

Should we still judge stallion fertility assuming that the BSE is a 'pass' or 'fail' exam?

Last, but not least, a consideration regarding the classification system for breeding stallions warrants some discussion. In food animals, any male that does not fulfill the criteria set for being considered as a potentially 'Satisfactory Breeder' is classified either as 'questionable' or 'unsatisfactory'. The 'questionable' category is used for males that might 'pass' a BSE after a certain interval if some parameters regarding physical health or semen quality can be improved. The 'unsatisfactory' category is used for males that definitively do not possess the physical, behavioral, and reproductive characteristics required to 'pass' a BSE, and that will not improve with time. For the stallion BSE manual, the criteria that define whether or not this individual will pass are:

• Demonstrates good libido, as indicated by short reaction time, to move freely, to find and mount the mare (or breeding dummy), to make intromission, and to promptly ejaculate semen free of urine and/or blood (either in the mare's vagina or an artificial vagina).

• The penis must be of normal size and shape and be free of lesions of an inflammatory nature.

• The bacteria recovered from the semen and 2 urethral swabs should be inconsistent in type and reduced in number after ejaculation. There should not be colonies of the organisms of Contagious Equine Metritis (Taylorella equigenitalis). In addition, multiple pure cultures, or an unexplained increase in colony count on the second ejaculate are considered reproductive tract infections and necessitate further investigation and clarification.

• There should not be an indication of equine infectious anemia as indicated by a negative Coggins test.

• There should be 2 scrotal testes and epididymides of palpably normal size, shape, and texture.

• The stallion should have the potential ability to ejaculate at least 1 x 10^9 morphologically normal, progressively motile sperm in the second ejaculate after 1 week of sexual rest, each month of the year.

If a stallion meets all these criteria, he is classified as a 'Satisfactory Prospective Breeder'. If he is borderline in 2 or more criteria, he is considered 'Questionable', and if he is very low in 2 or more criteria or has severe permanent shortcomings, he is considered 'Unsatisfactory'. Some of these criteria can be seen as an 'all or none' (e.g. those regarding the presence of venereal organisms in the reproductive tract and semen), the occurrence of cryptorchidism or aplasia of the internal reproductive organs (i.e. epididymis), or those mentioned above regarding the general health status of the stallion. Yet, rather than considering that a stallion passed his BSE because he fulfilled all the criteria previously mentioned, theriogenologists and general practitioners should see the BSE as a clinical tool instead of a 'checklist' to complete. If a stallion does not display adequate libido, has the presence of urine or blood in the ejaculate, presents some lesions in the penile integument, or his sperm number and quality is marginal, how can I approach those 'issues', and offer potential management to maximize this stallion's fertility? Certainly, many conditions that are noticed in breeding stallions can be easily diagnosed and managed, if not treated nowadays. Hence, we desist from classifying stallions based on these 3 categories when conducting a BSE, and we prefer to have a clear discussion with the owner/agent of that stallion, hoping to find the best clinical, ethical, and scientific approach to enhance stallion fertility. We hope the present document will serve as the basis for academic discussions among theriogenologists, general practitioners, academicians, owners, and agents, to encourage the use of the stallion BSE Manual, taking into consideration the new evidence that is daily published regarding male reproductive physiology and fertility.

The practitioner should also be concerned about the concentration of the sperm sample they have received (either cooled or frozen). Evaluation of sperm motility alone is an insufficient measure of sperm quality. Excellent sperm motility associated with low sperm numbers can result in low fertility.

Acknowledgments

Authors recognize the immense efforts of the committee that initially established the guidelines for the stallion BSE manual, as well as the dedication of many colleagues that dedicated their academic life to preserve and take to the next level what was envisioned by the pioneers in the discipline of stallion reproduction. Although many names came to our minds when thinking about stallion reproduction, the authors express their gratitude to their mentors and friends Bob Kenney, Terry Blanchard, and Dickson Varner. Other individuals who are not mentioned here were not ignored on purpose.

Conflict of interest

None to declare.

Funding

Legends Premier Stallion Season Auction, Texas A&M University

References

1. Koziol JH, Armstrong CL: Manual for Breeding Soundness Examination of Bulls, 2nd edition. Mathews, AL. Society for Theriogenology 2018.
2. Hurtgen JP: 1984. Manual for the Reproductive Examination of the Boar. Hastings, NE. Society for Theriogenology 1984.
3. Ott RS, Memon MA: Breeding soundness examinations of rams and bucks, a review. Theriogenology 1980;13:
155-164.
4. Purswell BJ, Althouse GC, Root Kustritz MV: Guidelines for using the canine breeding soundness evaluation form. Theriogenology Handbook, SA-C1 (8/92). 1992.
5. Kenney RM, Hurtgen J, Pierson R, et al: Manual for Clinical Fertility Evaluation of the Stallion. Hastings, NE. Society for Theriogenology 1983.
6. Blanchard TL, Love CC, Thompson JA, et al: Role of reinforcement breeding in a natural service mating program. Proc Am Assoc Equine Pract 2006;52:384-386.
7. Blanchard TL, Morehead JP, Ramsey J, et al: How to use planned multiple matings per estrus to improve pregnancy rates achieved by some aged subfertile Thoroughbred stallions. Proc Am Assoc Equine Pract 2008;54:364-366.
8. Varner DD, Love CC, Brinsko SP, et al: Semen processing for the subfertile stallion. J Equine Vet Sci 2008;28:
677-685.
9. Blanchard TL, Brinsko SP, Love CC, et al: Case study of processing and insemination techniques: attempts to improve fertility of an aged stallion with dilute semen of poor quality. J Equine Vet Sci 2012;32:5-11.
10. Blanchard TL, Brinsko SP, Love CC, et al: How to use testicular measurements for First-Season Subfertility Insurance considerations in Thoroughbred stallions. Proc Am Assoc Equine Pract 2008;54:374-379.
11. Thompson DL, Pickett BW, Squires EL, et al: Testicular measurements and reproductive characteristics in stallions. J Reprod Fertil Suppl. 1979;27:13-17.
12. Woods GL, Garcia MC, Kenney RM: Variations in testicular size of standardbred stallions. Proc Am Assoc Equine Pract 1980;26:117-121.
13. Blanchard TL, Varner DD: Evaluating breeding soundness in stallions – 5: predicting potential fertility. Vet Med 1997;815-818.
14. Pickett BW, Voss JL, Bowen RA, et al: Seminal characteristics and total scrotal width (T.S.W.) of normal and abnormal stallions. Proc Am Assoc Equine Pract 1987;33:487-518.
15. Love CC, García MC, Riera FR, et al: Evaluation of measures taken by ultrasonography and caliper to estimate testicular volume and predict daily sperm output in the stallion. J Reprod Fertil Suppl 1991;44:99-105.
16. Love CC: How to measure testes size and evaluate scrotal contents in the stallion. Proc Am Assoc Equine Pract 2014; 60:302-308.
17. Gebauer MR, Pickett BW, Voss JL, et al: Reproductive physiology of the stallion: daily sperm output and testicular measurements. J Am Vet Med Ass 1974;165:711-713.
18. Love CC, García MC, Riera FR, et al: Use of testicular volume to predict daily sperm output in the stallion. Proc Am Assoc Equine Pract 1990;36:15-21.
19. Varner DD, Schumacher J, Blanchard TL, et al: Diseases and Management of Breeding Stallions. Goleta, CA. American Veterinary Publications 1991. p. 104-105.
20. Thompson JA, Love CC, Stich KL, et al: A Bayesian approach to prediction of stallion daily sperm output. Theriogenology 2004;62:1607-1617.
21. Blanchard TL, Brinsko SP, Varner DD, et al: Progression of reproductive changes accompanying testicular dysfunction in aging Thoroughbred stallions: Case studies. Proc Am Assoc Equine Pract 2013;59:532-536.
22. Johnson L, Varner DD, Thompson DL Jr: Effect of age and season on the establishment of spermatogenesis in the horse. J Reprod Fertil Suppl 1991;44:87-97.
23. Johnson L, Thompson DL Jr: Age-related and seasonal variation in the Sertoli cell population, daily sperm production, and serum concentrations of follicle-stimulant hormone, luteinizing hormone, and testosterone in stallions. Biol Reprod 1983;29:777-789.
24. Kenney RM, Kingston RS, Rajamannon AH, et al: Stallion semen characteristics for predicting fertility. Proc Am Assoc Equine Pract 1971;17:53-67.

25. Bielanski W: The evaluation of stallion semen in aspects of fertility control and its use for artificial insemination. J Reprod Fertil Suppl. 1975;23:19-24.

26. Dott HM: Morphology of stallion spermatozoa. J Reprod Fertil Suppl 1975;23:19-24.

27. Stout TAE, Colenbrander B: Reproductive parameters of Draft Horse, Friesian, and Warmblood stallions. In: McKinnon AO, Squires EL, Vaala WE, et al: editors. Equine Reproduction. 2nd edition. Ames, IA: Wiley Blackwell: 2011. p. 1362-1366.

28. Blanchard TL, Varner DD, Love CC, et al: Management options for the aged breeding stallion with declining testicular function. J Equine Vet Sci 2012;32:430-435.

29. Voss JL, Pickett BW, Squires EL: Stallion spermatozoal morphology and motility and their relationship to fertility. J Am Vet Med Assoc 1981;178:287-289.

30. Dowsett KF, Pattie WA: Characteristics and fertility of stallion semen. J Reprod Fert Suppl. 1982;32:1-8.

31. Jasko DJ, Lein HD, Foote RH: Determination of the relationship between sperm morphologic classifications and fertility in stallions: 66 cases (1987 – 1988). J Am Vet Med Assoc 1990;197:389-394.

32. Jasko DJ, Little TV, Lein DH, et al: Comparison of spermatozoal movement and semen characteristics with fertility in stallions: 64 cases (1987 – 1988). J Am Vet Med Assoc 1992;200:979-985.

33. Love CC, Kenney RM: The relationship of increased susceptibility of sperm DNA to denaturation and fertility in the stallion. Theriogenology 1998;50:955-972.

34. Love CC: Relationship between sperm motility, morphology and the fertility of stallions. Theriogenology 2011;76:547-557.

35. Love CC, Noble JK, Standridge SA, et al: The relationship between sperm quality in cool-shipped semen and embryo recovery rate in horses. Theriogenology 2015;84:1587-1593.

36. Barrier-Battut I, Kempfer A, Becker J, et al: Development of a new fertility prediction model for stallion semen, including flow cytometry. Theriogenology 2016;86:1111-1131.

37. Brinsko SP, Spooner JA, Blanchard TL, et al: Relationships among morphologic characteristics and membrane integrity of stallion sperm. Clinical Theriogenology 2011;3:55-60.

38. Love CC, Varner DD, Thompson JA: Intra- and inter-stallion variation in sperm morphology and their relationship with fertility. J Reprod Fertil Suppl 2000;56:93-100.

39. Brinkerhoff JM, Love CC, Thompson JA, et al: Influence of mare age, pre-breeding mare status, breeding method, and stallion on first cycle pregnancy rates on a large commercial breeding farm. Anim Reprod Sci 2010; 121S:S159.

40. Love CC: The role of breeding record evaluation in the evaluation of the stallion for breeding soundness. Proc Soc Therio 2003;68-77.

41. Blanchard TL. The subfertile stallion. In: Samper JC, Pycock JF, McKinnon AO, editors: Current Therapy in Equine Reproduction. 1st edition. St. Louis, MO: Saunders Elsevier. p. 237-243.