How to Use Testicular Measurements for First-Season Subfertility Insurance Considerations in Thoroughbred Stallions

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1. Introduction

When owners retire a stallion to stud with the prospect of obtaining first-season subfertility insurance (sometimes called first-season congenital infertility insurance), a veterinary examination is required on behalf of the policy underwriter. In addition to assessment of general physical condition and health, heavy emphasis is placed on evaluation of genital normalcy, particularly the testes. Examinations include assessment of size, texture, orientation, and position of the testes, epididymes, and spermatic cords, freedom of movement of testes and their tunics within the scrotum, and appearance and thickness of the scrotum. Ultrasound or caliper measurements of the testes are also commonly requested. Based on these measures, the examining veterinarian is asked to place judgment on the likelihood that a stallion will achieve acceptable fertility (i.e., likely to achieve a minimum seasonal pregnancy rate of 60%) when booked to a defined number of mares. The veterinary examiner may also be asked to recommend the maximum number of covers per day (or per week) that should be allowed for stallions subjected to natural mating conditions. Typically, semen collection and evaluation are not permitted to determine if libido, erection, mating ability, ejaculatory function, sperm output, or semen quality are normal.

Previously, many veterinary examiners have passed judgment on potential fertility, book size, and mating frequency based on scant criteria, such as an examination limited to manual palpation of the testes. Research performed in the last two to three decades has provided a great deal of scientific information on normal testicular size, spermatogenic efficiency (including sperm-production rates), and sperm output in stallions. Although specific standards regarding minimum or threshold numbers of sperm that must be ejaculated during natural mating to maximize reproductive efficiency remain largely unstudied, the relationship of the above parameters to fertility in artificial insemination programs has been the subject of numerous investigations. Today's equine veterinarian has a

NOTES

Table 1. Total Scrotal Width (TSW), Measured by Calipers, Reported for Growing Horses[1]

Age 83 wk (19.1 mo) = 7.7 cm TSW
Age 96 wk (22.1 mo) = 8.8 cm TSW

substantial body of scientific data for reference when assessing young stallions in athletic training or just retired from a performance career. The purpose of this paper is to review and discuss the practical applications of some of these data and to provide a logical framework for justifying any conclusions drawn from testicular assessment. To this end, caliper and ultrasound measurement of scrotal width and testicular dimensions, calculation of testicular volume and predicted sperm output, and assessment of mating frequency and book size will be addressed.

2. Caliper Measurement of Total Scrotal Width

The total scrotal width (TSW); (greatest distance across both testes when the testes are gently positioned evenly and horizontally within the scrotum) obtained by the veterinary practitioner can be compared with mean values published for horses of different age groups (Tables 1 and 2).[1,2] Although this measurement is less useful for evaluating total testicular mass, it is an easy and repeatable measurement that is commonly used by equine practitioners throughout the world. Published values provide a framework for comparison of the stallion's scrotal width with that of a referenced population. Values published are from light-horse breeds but are not specifically for the Thoroughbred. A relationship between scrotal width and either semen quality or fertility was not determined in those reports. Additionally, horses included in those studies were not likely to have been in race training. However, in Thoroughbred or Standardbred stallions 3–6 yr of age that recently retired from racing and fail a breeding soundness examination because of low numbers of normal sperm, total scrotal width averaged 1.1 cm smaller than those that passed.[3,4] The measurement of total scrotal width can also be viewed as a quality-control value when second opinions are requested (e.g., after another examiner has reported individual testicular measurements that seem to differ substantially from expected values). Individual testicular widths, measured by calipers at the racetrack, have also been reported for Standardbred stallions of different ages (Table 3).

Table 2. Total Scrotal and Individual Testicular Widths (±SD), Measured by Calipers, for 43 Horses 2–16 yr of Age[2]

Age (yr)	TSW (cm)	LTW (cm)	RTW (cm)
2–3	9.6 ± 0.8	5.5 ± 0.5	5.3 ± 0.5
4–6	10.0 ± 0.7	5.7 ± 0.4	5.5 ± 0.5
>7	10.9 ± 0.7	6.1 ± 0.4	6.0 ± 0.5

TSW, total scrotal width; LTW, left testis width; RTW, right testis width.

Table 3. Testicular Widths, Measured by Calipers, in Standardbred Stallions at the Racetrack (±SD)[5]

Age (yr)	LTW (cm)	RTW (cm)	CTW (cm)
2	4.9 ± 0.64	5.2 ± 0.64	10.1 ± 0.64
3	5.1 ± 0.80	5.4 ± 0.81	10.5 ± 0.81
4	5.1 ± 0.82	5.3 ± 0.87	10.4 ± 0.85
5	5.1 ± 0.89	5.2 ± 0.82	10.3 ± 0.86
6	5.1 ± 0.97	5.4 ± 0.84	10.4 ± 0.91
7	5.2 ± 0.80	5.4 ± 0.68	10.6 ± 0.74
8	5.2 ± 0.74	5.2 ± 0.92	10.4 ± 0.83
9	5.3 ± 0.75	5.5 ± 0.70	10.8 ± 0.73

When the authors subsequently compared values for 5- to 9-yr-old stallions residing at breeding farms with those of the same age group but still located at the race track, CTW averaged 1.32 cm greater for those at a breeding farm.

LTW, left width; RTW, right width; CTW, combined testes width (left plus right).

3. Value of Testicular Volume Measurement

Estimates of testicular volume can be calculated from caliper or ultrasound measurements of three parameters (length, width, and height) for each individual testis. The authors believe that properly taken ultrasound measurements of testicular dimensions are more accurate than caliper measurements, because only testicular parenchyma is included in the measurement determined ultrasonographically. The formula used for estimation of testicular volume is that given for the volume of an ellipsoid, because it closely resembles the three-dimensional shape of a stallion testis.[5,6]

$$4/3\ (\text{length}/2)(\text{height}/2)(\text{width}/2)$$

or

$$0.5233 \times \text{length} \times \text{width} \times \text{height}$$

The computed testicular volume can then be compared with values published for horses of different age groups (Fig. 1).[7] Testicular size is also known to vary with season of the year (particularly between winter and summer), because stallions are seasonal breeders.[7] This seasonal variation in testicular size is greater in older (>5 yr) stallions than in younger stallions.

For young horses (i.e., ≤3 yr of age) with small testes, comparisons can also be made to published data on the relationship between testicular size and onset of adult spermatogenic efficiency. In this regard, the size of the growing testis is better correlated with normal (adult) spermatogenic efficiency than with age of the horse. For example, each individual colt testis must increase to 60–80 ml in size (120–160 ml for both testes combined, which corresponds to a minimum total scrotal width (TSW) of 8.2–8.4 cm) before they are capable of producing sperm with the efficiency (sperm production per

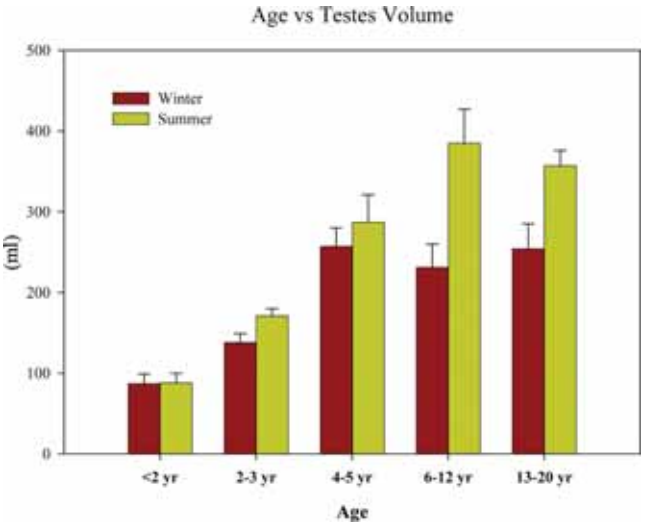

Fig. 1. Mean total testicular volume for horses of differing age groups obtained at an abattoir during winter or summer.[7] Weights of individual testes were doubled to represent two testes and converted to volume, (ml).

gram of testis) typical of adult stallions (Fig. 2).[7,8] This adult level of spermatogenic efficiency is generally achieved at 3 yr of age.[7,8] No firm predictions can be made as to whether or not the small testes present in a colt will grow to typical adult size. Therefore, when each testis is <80 ml, caution should be exercised in making predictions about

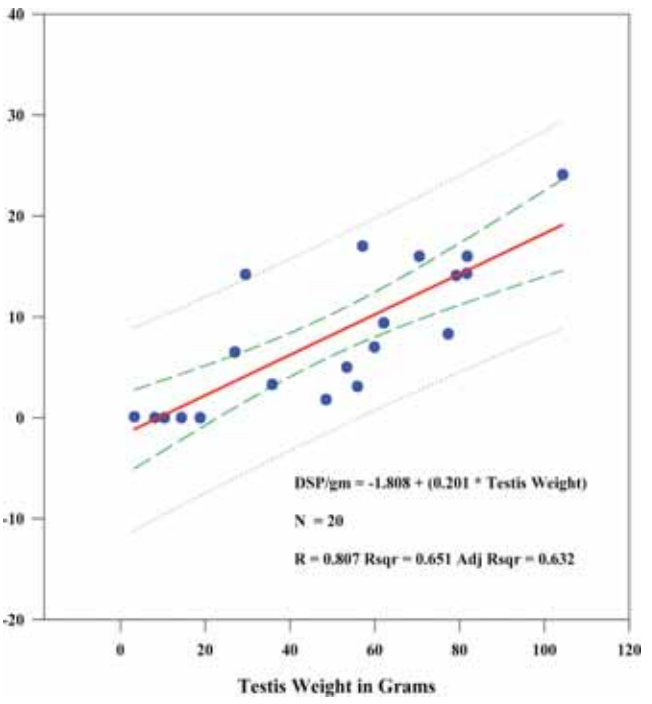

Fig. 2. Normal spermatogenic efficiency (e.g., daily sperm output [DSO]/g parenchyma) for adults is not reached until each testis reaches 60–80 g (~60–80 ml).[8]

Table 4. Calculation of Mean Daily Sperm Production (DSP) in Millions of Sperm per Milliliter of Testis During Breeding and Non-Breeding Seasons in Stallions >3 yr of Age

Season	No. Stallions	Mean (±SD) DSP/ml of Testis
Breeding	43	16.52 ± 3.22 million/ml
Non-breeding	45	12.87 ± 3.69 million/ml

As an average, DSO (daily sperm output in ejaculates) = 0.87 × DSP (daily sperm production in testes).[10] Weights were converted to volume.

sperm output. Additionally, when stallions >3 yr of age have testes smaller than this value, they are probably not producing sperm at a rate typical of normal adult testes.

4. Predicting Daily Sperm Output From Testicular Volume Computation

If one assumes that testicular parenchyma in a given horse is normal (i.e., producing sperm at a normal adult rate), the total number of sperm that a horse's testes can produce (Table 4)[9] and the amount that the horse can then ejaculate on a daily basis[5,6] can be estimated from the calculated total testicular volume of testes obtained by ultrasound or caliper measurements. Daily sperm output (DSO) is calculated by using the following regression equation:

$$\text{DSO (billions of sperm per day)} = 0.024 \times \text{total testicular volume in ml (TTV)} - (0.76 \text{ to } 1.26)^{5,6}$$

Predicting the DSO from testicular size allows the veterinarian to estimate the potential number of mares that a stallion can breed on a per-day basis. For a sexually active horse, DSO represents the total number of sperm available for ejaculation within a 24-h period (regardless of number of ejaculates within that day). Assuming that matings are evenly spread throughout the day, dividing the DSO by the number of covers in a day should approximate the number of sperm available in each ejaculate. Thus, DSO can be used to predict if a given stallion can be used at a certain mating frequency and if he could be expected to efficiently serve a proposed number of mares in his book.

5. Making Judgments About Potential "Book" Size

An insurance company often requests an opinion on a proposed number of mares that a given stallion could effectively serve in a breeding season (i.e., a certain-sized mare "book"). They may also ask if a stallion could achieve adequate pregnancy rates when mated a given number of times per day (or per week). To address this issue, the veterinarian can estimate the number of covers required to service a projected book of mares in a breeding season. This total number of projected covers can be compared with a conservative estimate of the number of covers

Table 5. Example of Estimated Number of Covers per Day That Can Be Safely Accommodated by Stallions With Different Testicular Sizes

Testicular Volume (ml)	Range in Predicted DSO	Number of Covers/Day
200	3.54–4.04	2.36–2.69 (2 and occasionally 3)
250	4.74–5.24	3.16–3.49 covers/day (3)
300	5.97–6.44	3.96–4.29 (4)
350	7.14–7.64	4.76–5.09 (5)

This assumption is based on a presumed requirement of 1.5 billion total sperm per cover. If a lesser number is required per cover, correspondingly more covers would be available per day.

DSO, daily sperm output in billions.

to which a stallion could be subjected in a given day (that should still yield adequate sperm numbers in the ejaculates) multiplied by the number of days in the season. In a natural service program, the authors' believe 1.5 billion is a conservative sperm number per ejaculate that should yield an acceptable pregnancy rate (provided that semen quality is normal). The estimated sperm number per ejaculate is calculated as the quotient of the predicted DSO (dividend) and the number of covers per day (divisor). An example of testicular volume versus estimated number of covers per day is provided in Table 5.

Although the number of covers required for a given book size is highly variable (being dependent on management level, size of the book, and fertility of both the stallion and the mares in his book), data (Fig. 3) obtained using Thoroughbred stallions provides an expected range in number of covers required for the season. The 95% confidence interval for the total number of covers (including doubles) required to service a proposed book size is determined from the graph, which provides the range in number of covers required. Most Thoroughbred stallions service the majority of the mares in their book in 90–120 days (i.e., 87.5% of all stallion covers during a season occur by the end of May; Fig. 4). The number of covers per season can be estimated by multiplying the 90–120 days of active breeding by the number of covers per day that the stallion should be able to safely provide. If the calculated maximum 90–120 day number of covers exceeds the range in number of covers required for the given book size determined from the graph, then the proposed book size would be considered acceptable. For example, if the predicted DSO is sufficient for 3 covers/day, the stallion should be able to provide a range from 270 (e.g., 90 days × 3 covers/day) to 360 (e.g., 120 days × 3 covers/day) total covers during the season. If the proposed book was 150 mares, the 95% confidence interval of the graph indicates a range of 215–280 covers would be required. Because the calculated total available covers from the 90–120 days of active breeding at 3 covers/day (270–360 available covers) exceeds the 215–280 covers

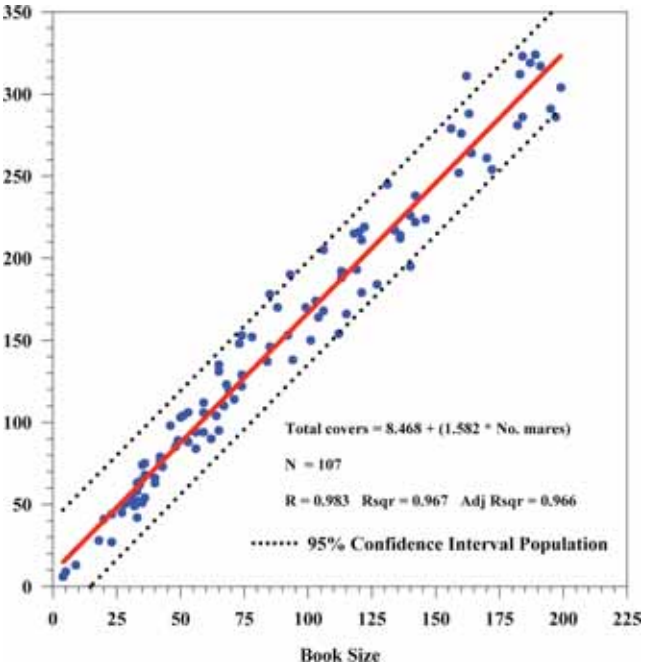

Fig. 3. Total covers (including doubles) expended during the breeding season in Thoroughbred stallions; data includes 107 seasons and 29 Thoroughbred stallions with pregnancy rates per cycle ranging from 40 to 75%.

required to service 150 mares, the book size would be considered acceptable.

A simpler method for predicting if a stallion with a given testicular size can safely handle a certain book of mares is to use data on the actual number of days in the season in which Thoroughbred stallions with varying book sizes provide 0–5 covers/day (Table 6). Again, the stallion's predicted DSO is used to justify the maximum number of covers per day as described above. Then, the largest book size that requires that maximum number of covers be performed in a day can be determined from the table.

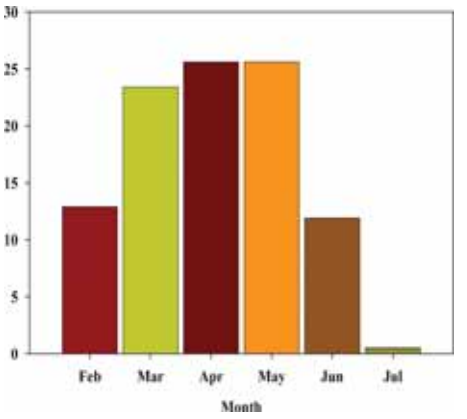

Fig. 4. Distribution of covers (n = 16,080) by Thoroughbred stallions (n = 29) during the breeding season (northern hemisphere).

Table 6. Number of Days in the Entire Breeding Season in Which Zero to Five Covers Are Required From Thoroughbred Stallions With Varying Book Sizes (Increments of 10 mares)

		Days in Season With Zero to Five Covers (Includes Doubles)					
Book Size	N	0	1	2	3	4	5
0–19	4	122	14	0	0	0	0
20–39	19	92	36	7	1	0	0
40–49	9	76	45	13	2	0	0
50–59	9	62	52	20	2	0	0
60–69	8	58	50	24	5	0	0
70–79	6	49	50	27	9	0	0
80–89	4	35	55	36	8	1	0
90–99	4	31	59	36	11	0	0
100–109	5	30	53	42	10	1	0
110–119	7	35	43	36	20	3	0
120–129	5	29	45	40	19	5	0
130–139	4	25	40	43	22	7	0
140–149	5	24	44	41	21	7	1
150–159	2	19	36	38	26	17	2
160–169	4	17	32	37	29	22	0
170–179	2	27	29	37	31	13	1
180–189	6	13	30	35	33	25	2
190–199	4	15	27	37	33	23	2

The table was constructed using 12 yrs of data from 29 stallions having a total of 16,080 covers. Blanchard and Brinsko, unpublished data.

For example, if a DSO of 4.5–5 billion sperm is predicted from the ultrasound measurements, the veterinarian would be justified in suggesting 3 (and occasionally, 4) covers/day be allowed. Using table 6, it can be seen that until the book size exceeds 149 mares, stallions should have ≤7 days during the season in which they would have to cover 4 mares/day. Therefore, the book size of 150 mares would be at the upper safe limit for a stallion with a predicted DSO of 4.5–5 billion sperm/day.

6. Precautions

Both the veterinarian and the insurance company must remain cognizant that all the above calculations are derived strictly from testicular measurements. Assumptions are, therefore, made that the stallion will have normal libido and mating ability that, if compromised, can severely limit fertility because of factors other than level of sperm production and output. Because semen collection/evaluation is not permitted before writing the insurance policy, there is no assurance that the stallion is capable of achieving a normal erection, mounting the mare, inserting the penis fully into the vagina, thrusting, or ejaculating normally. Without semen evaluation(s), it is not possible to determine if the assumption of normal sperm production and semen quality were justified. Finally, true fertility can only be determined by mating an adequate book of well-managed, reproductively normal mares.

Table 7. Testicular and Scrotal Widths (±SD) in 2-yr-old Standardbred Stallions on the Racetrack With or Without the History of "Drug" (e.g. Anabolic Steroid) Administration[5]

Medications Given	LTW (cm)	RTW (cm)	CTW (cm)
No	5.0 ± 0.7	5.4 ± 0.5	10.4 ± 0.6
Yes	4.6 ± 0.7	4.7 ± 0.7	9.2 ± 0.7

LTW, left testis width; RTW, right testis width; CTW, combined testes width.

7. Some Indications for Reexamination at a Later Date

For young stallions whose testes are measured while still in race training or after recent retirement to a breeding farm (Table 7),[10] the testes may be too small to justify either insuring the stallion for first-season subfertility or for planning a large book of mares. Causes of abnormally small testes can include testicular hypoplasia (congenital), past illnesses (particularly those causing fever), swelling of the scrotum, excessive cortisol release from chronic stress, rigorous training, and drug administration (e.g., anabolic steroids). Information regarding these conditions should be disclosed on the trainer's declaration. In some cases, however, the information may be lacking or the trainer may not be forthcoming with a precise history. Unless specifics are given that would suggest an insult to the testes occurred, it becomes difficult to state with any certainty that the testes are likely to increase in size. Testicular recovery (i.e., growth to normal size and sperm production) from thermal injury (e.g., fever or scrotal swelling) can take 2–5 mo.[11] Recovery of testicular size after prolonged anabolic steroid administration may also take several months. It is possible that some drugs given to growing horses during the peri-pubertal period could have permanent deleterious effects on the developing testes. Therefore, if a recently retired stallion has testes too small to acquire first-season subfertility insurance, reexamination of the horse at 1- or 2-mo intervals is recommended to determine if testicular size is increasing.

The same reasoning is followed when testicular size is not sufficient to support a proposed large book of mares (or proposed mating frequency). An insurance company may impose a restriction on the maximum number of covers permitted per day (or per week). Reexamination at a later date may reveal a sufficient increase in testicular size to support expanding the stallion's book (and maximum mating frequency). Finally, there is no substitute for collecting several ejaculates to determine the actual DSO and to analyze semen for percentage and number of normal sperm. Many laboratory tests can be used to assess normalcy of the testes and sperm, which then allows for a more accurate assessment of number of mares that can be safely bred at that time. It is possible that semen collection and evaluation may one day become an in-

tegral part of the veterinary examination required to obtain first-season subfertility insurance. Until that time, however, the veterinarian will have to make a recommendation on whether or not a stallion should qualify for first-year subfertility insurance and whether or not the stallion can be expected to handle a certain book size or mating frequency based strictly on physical examination and measurement of testicular size.

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How to Use Testicular Measurements for First-Season Subfertility Insurance Considerations in Thoroughbred Stallions. Terry L. Blanchard, DVM, MS, Diplomate ACT; Steven P. Brinsko, DVM, MS, PhD, Diplomate ACT; Charles C. Love, DVM, PhD, Diplomate ACT; and Dickson D. Varner, DVM, MS, Diplomate ACT.Â Testicular measurements and reproductive characteristics in stallions. J Reprod Fertil 1979;27(Suppl):13â€“17. 3. Blanchard TL, Varner DD.Â Use of testicular volume to predict daily sperm output in the stallion, in Pro-ceedings. 36th Annual American Association of Equine Practitioners Convention 1990;15â€“21. 7. Johnson L, Thompson DL Jr. Subfertility and infertility are not the same. Learn about their differences, as well as what to do if you may have subfertlity issues.Â Subfertility is a delay in conceiving. Infertility is the inability to conceive naturally after one year of trying. In subfertility, the possibility of conceiving naturally exists, but takes longer than average. In infertility, the likelihood of conceiving without medical intervention is unlikely. According to research , most couples are able to conceive spontaneously within 12 months of having regular unprotected intercourse. Background Drugs to stimulate ovulation have been used to treat subfertility since the early 1960s. There is uncertainty about the safety of these drugs and their potential risk of causing cancer. Moreover, it has already been shown that infertility itself increases the risk of ovarian cancer.Â A cumulative analysis of 12 case-control studies from the USA revealed increased risk of ovarian cancer in women using fertility drugs, and this risk was higher in nulliparous women (women who have not given birth) when compared to multiparous women (women who have given birth to more than one child).