The role of breeding record evaluation in the evaluation of the stallion for breeding soundness

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Introduction: The stallion breeding soundness evaluation is a commonly used procedure to. The guidelines outlined by the Society for Theriogenology are also used for diagnostic purposes when a stallion that is currently used for breeding purposes encounters difficulty in rendering sufficient mares pregnant based on the expectations of the client as well as determine the potential fertility of a stallion with no prior breeding experience. In the latter case, the stallion has no prior fertility history and therefore the clinician must make recommendations almost solely based on semen evaluation and physical/reproductive examination. However, in the former instance, the clinician has the opportunity to gather and evaluate the fertility of the stallion in the weeks and years leading to the suspected case of reduced fertility.

There are many reasons why a stallion may be identified as having reduced fertility or being subfertile including the following:

1. **Testicular health** is compromised resulting in a reduction in sperm quality such that the threshold level of normal sperm needed to assure an adequate level of fertility is not attained. These types of insults include testicular trauma, heat stress, systemic (hormones) substances that affect spermatogenesis as well as testicular changes that occur as a result of old age.
2. **Non-stallion factors** include those influences that alter the fertility of a stallion below what it would have been if the stallion had been bred by natural cover and are not directly related to the ability of the stallion to produce sperm of sufficient quality and deliver it to the mare at the proper time for maximum fertility.

Natural cover: As a reference point in this article the natural cover or natural breeding situation will be considered as having the potential for attaining the highest level of fertility and therefore the conditions surrounding its used will be considered the gold standard to which all other breeding methods will be compared. When the effects of management and mare influences are of interest, natural cover conditions minimize the effect of these influences. It cannot be assumed that semen-handling techniques, such as the use of artificial insemination, maximize the fertility potential of a stallion and do not play a role in cases of reduced fertility.

Historically, stallions have been bred using either natural cover or using artificial insemination. In either case, the stallion was bred with fresh sperm or sperm that had not been altered by cooling, freezing, or long-term storage. In the last 15-20 years the increased use of shipped and frozen sperm has increased dramatically as breed registries have allowed its use. Coincidental with the marketing of these “semen technologies” has been an increase in the incidence of reduced fertility in some stallions that were “fertile” prior to the application of these techniques.

While it is not clear what factor (s) account for this type of reduced fertility, non-stallion factors may magnify fertility problems that already exist. It is helpful for the clinician to appreciate some of the advantages of natural cover/breeding over artificial insemination when approaching these cases of reduced fertility. Some advantages include the following:

1. Exposure of mares to a greater than threshold level of normal sperm. Therefore each mare is assured of having adequate sperm numbers deposited in their uterus. This is oftentimes assumed when AI is used, but cannot be accommodated when stallions are overbooked and semen is divided to accommodate all semen requests rather than assure that adequate sperm numbers are provided to the customer.
2. Mares are in standing estrous. Almost by definition this must occur if the stallion is going to safely mount and be able to breed a mare by natural cover. This assures that no mares are bred that are not in heat or bred due to client pressure to breed them when the mares may not be ready.
3. Semen is not handled/processed and therefore there is no possibility that a stallion with good quality sperm will be compromised by poor handling technique or be exposed to toxic substances.
4. Breeding management of intensively managed Thoroughbred mares bred by natural cover is monitored such that ovulation is closely timed with breeding in an attempt to assure that the mare is only bred once, close to ovulation, to minimize the number of times that a stallion will breed a mare during one estrous period. It is uncommon for an intensively managed Thoroughbred stallion to have more than 10% of estrous cycles in which mares will be bred more than once. Breeds in which artificial insemination (AI) is used will breed a mare more often when she is in estrus, because of the ease of doing so. While AI allows the breeder to theoretically breed more mares with a single ejaculate, there are instances in which a popular stallion does not have sufficient sperm to accommodate all the mares that must be bred in a particular time period. In most cases all mares will receive semen, but each inseminate will be a less than threshold level required for optimal fertility. The Thoroughbred industry appears to be able, in many cases, to breed similar numbers of mares and are able to do so by reducing the number of times the average mare is bred during the estrus period when compared to those breeds that use AI.
5. Fewer management systems involved in the breeding process. The widespread use of shipped and frozen semen has increased the number of individuals involved in the breeding of a mare and therefore, fertility can be limited by the abilities of the weakest link. Prior to the use of shipped and frozen semen most breeding operations had both the mares and stallion on the same farm under a common management or mares were shipped to the stallion. In either case the mares was bred with fresh sperm that was at the most only a few hours since ejaculation. Shipped and frozen semen has enabled more individuals to become involved in the breeding industry but has also resulted in a dilution of the expertise. In addition, the cooling and shipping or freezing of sperm has stretched the abilities of some stallions' sperm to remain at a level of fertility that accommodates commercial viability. While these marginal stallions can be managed to be commercially fertile when fresh semen is used, their fertility may be dramatically reduced when the sperm is processed.

In some of our studies at Texas A&M University we are interested in describing and defining

non-sperm factors and the role they play in understanding and diagnosing the primary cause(s) of reduced fertility in the stallion and mare. We hypothesize that semen quality accounts for 20-40% of the variability in the fertility of the average stallion. This assumes that the stallion does not have a dramatic sperm-limiting factor such as extremely poor semen quality or very low sperm numbers. This hypothesis suggests, therefore, that 60-80% of a stallions fertility can be explained by non-sperm factors, broadly categorized as management and mare factors. This presentation will deal primarily with the evaluation of these non-sperm factors and the impact they have on stallion fertility endpoints. The data described in this presentation has been summarized using a self-designed computer program. While some fertility endpoints can be easily calculated using pen and paper, most of the values described are time consuming unless computer methods are utilized. Outlined below is a series of endpoints that we use to describe non-stallion factors when evaluating a Thoroughbred stallion

Evaluation of overall fertility

Historically, the overall fertility of a stallion can be described by determining the *seasonal pregnancy rate* and *overall cycles per pregnancy*. These endpoints account for the combined effects of the stallion, mare, and management and are usually the first endpoints identified by clients when a case of reduced fertility is brought to the clinicians attention.

1) **Seasonal pregnancy rate -** this parameter is based on the number of mares diagnosed pregnant at a particular point in gestation divided by the total number of mares in a stallions book. This endpoint is a very important economic endpoint, but is not a sensitive indicator of a stallion's fertility because it does not reflect the total number of cycles that a mare is bred to achieve the pregnancy. A stallion can achieve a relatively low pregnancy rate per cycle yet end the season with a seasonal pregnancy rate similar to a stallion achieving a relatively high pregnancy rate per cycle; the only difference is the stallion with a relatively low pregnancy rate per cycle must breed the mares in his book more times during the season to reach the same seasonal pregnancy rate. Pregnancy rate is the fertility endpoint commonly talked about, but is not a sensitive endpoint and does not describe the source of reduced fertility.

   There are several factors that can alter seasonal pregnancy rate:

   a. The point in time seasonal pregnancy rate is determined (i.e., during the breeding season, shortly after the breeding season, or many months after the breeding season). As the point in time approaches the next breeding season the value will more closely approximate the *foaling rate* and will decrease as the effects of early embryonic loss and abortion are taken into account.

   b. The number of estrous cycles that a mare was bred. It is common for the stallion manager/owner to *add mares on to a stallion's book* as the end of the breeding season approaches. Adding mares on to a stallion's book is often done when mares are switched from one stallion to another for a variety of reasons. These mares are included in the total number of mares that the stallion breeds, but in reality are given fewer opportunities to become pregnant (usually only 1 cycle) than other mares in the stallion's book. If

mares added on to a stallion's book near the end of the breeding season do not become pregnant, the seasonal pregnancy rate for that stallion will be lower than expected. Therefore, if a stallion is presented because of a *low seasonal pregnancy rate,* it is important to determine that all mares were bred during an adequate number of estrous cycles to have a reasonable chance of becoming pregnant.

c. Inclusion or exclusion of mares that exhibit early embryonic death (EED). The assumption can be made that the majority of embryonic deaths are due to mare and not stallion factors. If an accurate seasonal pregnancy rate figure is to be created to describe the stallion's inherent fertility, all diagnosed pregnancies (which indicate that the stallion was able to accomplish fertilization in those mares) should be included in calculating the seasonal pregnancy rate. Yet, keep in mind that counting an embryonic death as a pregnancy when figuring seasonal pregnancy rate is of no economic relevance (i.e., stud fees will not be transferred). If embryonic deaths are included as pregnancies in calculating seasonal pregnancy rate, an inflated economic value for the seasonal pregnancy rate is created.

2) Cycles/pregnancy (or pregnancy rate/cycle) - this measure is a more sensitive indication of a stallions fertility because it measures how *efficient* a stallion is in establishing pregnancies and can be determined by counting all estrous cycles from all mares that a stallion has bred and dividing it by the number of pregnancies. The assumption is made that all mares were in normal estrus at the time of breeding and were bred near the time of ovulation. This assumption is not always correct, especially for breedings by artificial insemination. No doubt many mares are bred artificially when either not in estrus or near to the time of ovulation.

Some mares are ***doubled,*** and are bred more than once during an estrus period. On well-managed Thoroughbred farms, the *double-rate* rarely exceeds 10%. A double should not be counted as a separate cycle.

Evaluation of non-stallion factors

There are several broad categories that record analysis should evaluate as well as the endpoints that can be used to evaluate those categories:

1) ***Influence of mare type on pregnancy outcome*****-** mare type refers to the reproductive status of the mare coming into the breeding season and is commonly divided into maiden, barren, and foaling mares. Recently, the barren mares have been further divided into mares that were *not bred* the previous season; mares that became pregnant the previous and subsequently *slipped* or lost their pregnancy; in addition to those mares that had adequate opportunity to become pregnant the previous year, but did not become pregnant and are truly *barren*.

a) *Barren.* This group contains nonpregnant mares coming into the breeding season of interest. Mares in this group will usually have lower fertility than the other groups. There are several reasons why a mare might be barren, including:
   i) *Not bred the previous season*. This can occur because the owner simply decided not to breed the mare, or the mare may have foaled late in the previous season and did not

have ample opportunity to become pregnant. Mares barren due to these reasons have normal fertility.

ii) *Subfertile*. These mares have intrinsic fertility problems that contributed to failure to become pregnant the previous breeding season, and are commonly older than the rest of the mare population. In most cases, the subfertile mares will account for the majority of the mares classified as barren.

iii) *Slipped*. Mares that have aborted during the previous breeding season. Some managers and computer programs use the term *slipped* instead of the term aborted.

b) *Foaling*. Mares that produced a foal in the current breeding season and will be rebred during the same season. One should expect high fertility in this group of mares as they recently conceived and carried a foal to term. This group may exhibit reduced fertility if a predominant proportion of the mares foaled late in the breeding season, thus having only 1 estrous cycle available for breeding. Fertility in this group may also be reduced if some event (injury/illness to the stallion) prematurely shortens the breeding season.

c) *Maiden*. Mares that have never been bred. Mares in this group are generally young; however, occasional maiden mares are older due to owner election not to breed when young (usually due to a continuing performance career). Older maiden mares are generally less fertile than young maiden mares, which typically are of high fertility. Mares that are recently retired from strenuous performance careers may not be cycling regularly when they first become available for mating (typically in February) and thus may require more breedings to become pregnant. However, since maiden mares are generally available for breeding early in the season, their chances of becoming pregnant are high.

Evaluation of mare type- The number of each mare type and their percentage contribution to the book can be determined. In general, foaling mares account for 50-60% of the book with barren (20-30%) and maiden mares (10-20%) accounting for the rest. In unusual cases these ratios are different and can affect SPR and C/P.

Cycles/pregnancy (pregnancy rate/cycle) for individual cycles – since mares that do not become pregnant to their first cycle tend to get rebred, determining the pregnancy rate for individual cycles (1, 2, 3, etc) is valuable. Ideally, if all mares are of equal and high fertility, the pregnancy rate achieved in mares bred during the last estrous cycle should be the same as that in the first estrous cycle - and these pregnancy rates would accurately reflect the stallion's fertility. However, since individual mare fertility varies, actual pregnancy rates for each successive estrous cycle of breeding are seldom the same. Mares that are subfertile tend to take more estrous cycles to become detectably pregnant. The effect of poor quality mares is suspected when a dramatic decline in pregnancy rate occurs as cycle number increases (i.e. the 2^{nd} cycle PR = 50% and the 3^{rd} cycle PR = 30%).

2) ***Mare age-*** the age of the mare population to which a stallion is bred can be an important influence in that stallions fertility. As mares age and approach 12-14 years of age, as a population, fertility declines. Some stallions may accumulate a larger group of mares that are older than that of the average stallion and will experience reduced fertility usually because

these mare tend to be barren (see example 1 above).

3) ***Opportunity to become pregnant***- The ability of a mare to become pregnant is influenced, to a certain degree, simply by the number of estrus cycles that she is bred regardless of the intrinsic fertility of the stallion. A reproductively normal mare that is bred once to a stallion that has a 50% per cycle pregnancy rate has a 50% chance of getting pregnant; if bred two cycles she has a 75% chance; if bred 3 times, she has an 87.5% chance of getting pregnant. Opportunity is less important when highly fertile stallions (i.e. high per cycle pregnancy rate) are bred because they require fewer cycles to render a mare pregnant, making opportunity more important as the fertility of a stallion decreases.

   Evaluation of opportunity can be determined using several estimates:

   a) The *average day of first breeding* for each mare type. This is determined by averaging the first day of breeding for all mares in each mare type. The intent of this value is to determine when mares in each group started to be bred in the breeding season. Maiden and barren mares have the potential to start February 15th, since they enter the breeding season not pregnant. In contrast, foaling mares must wait until they foal before they can be rebred and therefore, as a group will start the breeding season later. As a general rule the average day of first breeding for maiden and barren mares should be sometime in the month of March, whereas, foaling mares tend to start in mid to late April.
   b) *Cycles/mare* for each mare type- this endpoint determines the number of estrous cycles that each mare was bred. Maiden and foaling mares approximate 1.3-1.6 cycles/mare, with barren mares around 2.0. Maiden mares are low because they tend to be a highly fertile group and therefore get pregnant at an efficient rate and simply don't need to be bred more; whereas foaling mares are low because they must foal before they can be bred. In the latter case, the foaling mares have a lack of opportunity in some cases because the end of the breeding season occurs before they can be bred again. This can result in a low seasonal pregnancy rate in foaling mares, not because the stallion is at fault, but because the mares were not bred enough. Interestingly in some cases barren mares will have a higher pregnancy rate than foaling mares, not because they are more fertile, but because they were bred more.

4) ***Overbreeding***- stallions may possess highly fertile sperm characteristics (excellent sperm motility, high percent of morphologically normal sperm), but may be limited by the ability to deliver a threshold level of normal sperm for the number of mares in his book. The effects of overbreeding are usually manifest as a reduction in the *efficiency* (cycles/pregnancy) by which a stallion renders his mares pregnant, which, if dramatic enough will result in a reduction in seasonal pregnancy rate.

   a) *Daily pregnancy rate based on the number of mares bred/day*- this value compares the fertility based on the number of mares that a stallion breeds per day. Theoretically, a stallion that breeds only one mare/day should deposit more sperm in the uterus than when he breeds 2, 3, or 4 mares/day. This value simply compares the overall pregnancy rate on days when 1 mare is bred vs. 2 vs. 3 vs. 4, etc., and determines whether pregnancy rate declines as more mares are bred/day.

b) Pregnancy rate based *on the order* in which a mare is bred during the day. This value is more sensitive than the daily pregnancy rate, because it specifically compares the pregnancy rate of those mares that were the stallions first cover of the day, with the mares that were the 2nd, 3rd, 4th etc. If a stallion exhibits overbreeding pregnancy rates will decline on the latter covers.
c) Decline in pregnancy rate coincidental with a *rise in the number of mares bred in a week*. To identify this phenomenon pregnancy data must be charted (see figures xxx) along with the number of mares bred in the week prior to the day the pregnancy data was gathered. The intent of these values is to determine if a stallions ability to deliver adequate numbers of sperm is depleted after a certain number of mares bred. In contrast to a. an b. above, sperm numbers may only fall below a threshold level after an extended number of ejaculates (i.e. >4).

While there are many factors involved in the breeding process, perhaps the most critical is realizing that mares need adequate exposure to the stallion for high fertility to be achieved (i.e., a 100% pregnancy rate per cycle is not achievable, so mares not becoming pregnant on first service must be bred a sufficient number of times to afford a realistic opportunity to become pregnant during the season). To illustrate this principle, if a stallion achieves a 50% pregnancy rate per cycle, mares must on average be bred at least two estrous cycles to yield a 75% seasonal pregnancy rate. Yet, a 75% seasonal pregnancy rate would be considered to be low on well-managed breeding farms. Evaluation of breeding records will reveal whether the low seasonal pregnancy rate is simply due to insufficient exposure to the stallion, or if other factors explain the low seasonal pregnancy rate.

Evaluating the effect of breeding frequency on fertility

One factor that will modulate fertility during the breeding season is the frequency of stallion use. For a farm that is using natural cover, the *breeding frequency* represents the number of times a stallion is bred in a given time period (i.e., the number of times bred in a day or week). On farms where artificial breeding is used, the *breeding frequency* represents the number of mares bred with an individual ejaculate. No stallion will have mares presented for breeding at even intervals throughout the breeding season. Therefore, pregnancy rates achieved by the stallion when used for different breeding frequencies can be used to evaluate whether overuse of the stallion has occurred (i.e., whether he has been bred too frequently for ejaculates to contain sufficient numbers of sperm to effect good fertility). Use of breeding frequencies to evaluate this phenomenon is presented in Figures 18-1 thorough 18-4, and relies on the following parameters:

1. *Number of mares bred in last week* - identifies the number of mares bred in the week previous to the day in question. This parameter is an evaluation of the long-term effect of frequent breedings, with the intent to determine whether there is a threshold breeding frequency above which fertility of the stallion declines. This phenomenon occurs in many stallions; if periods of too high breeding frequency are numerous during the season, reduced fertility becomes apparent.

2. *Number of mares bred the previous day* - identifies the number of mares bred (either by natural or artificial breeding) on that day.

3. *Pregnancy differential* - for each date every mare is bred, a pregnancy score is given (+1 if pregnancy results, -1 if no pregnancy results). The scores are summed for the week previous to a particular date, and graphically indicates the fertility of a stallion for the previous week.

4. *Cumulative pregnancy value* - this value represents the summed pregnancy scores (+1 or –1) for the entire breeding season leading up to the date of interest. The zero line represents a 50% pregnancy rate per cycle.

To illustrate the effects of breeding frequency , the reader is referred to Figure 1. The arrow (March 7th) represents the date for recording the following breeding frequency values: number of mares bred in last week = 10; number of mares bred previous day = 3; pregnancy differential = 3; and cumulative pregnancy value = 17. Interpretation of this data reveals that on March 7th: there were 10 mares bred in the previous week, 3 more mares became pregnant than were nonpregnant (pregnancy differential), and for the breeding season on this date there are 17 more mares pregnant than nonpregnant.

5) ***Seasonality of pregnancy***- the breeding season officially starts February 15th and extends to the end of June and early July and therefore includes both non-physiologic (short day lengths) and physiologic (longer day lengths) breeding season. The fertility of a stallion can vary considerably on a month-to-month basis based on a number of factors. Ideally mares that are anticipated to be bred early in the breeding season have been exposed to long day length conditions using artificial lighting to initiate normal cyclicity early in the year. Sometimes while mares may have been exposed to lighting there may be an inadequate response, resulting in prolonged cycles or a transitional-like state. This will result in reduced fertility early in the breeding season. Another cause of a reduction in fertility early in the breeding season is a large book of barren mares and/or maiden mares that are in poor reproductive shape. If the barren mares group is extremely poor they will continue to be bred and remain open until the end of the season and therefore will result in poor fertility late in the breeding season as well.

Table 1. Fertility endpoints for a group of Thoroughbred stallions showing overall pregnancy and mare type values.

Overall pregnancy	n	average	st. dev.	minimum	maximum
# mares bred	34	116.7	49.5	56	219
total cycles	34	185.4	79.2	88	348
# pregnant	34	92.4	38.1	45.0	179.0
% pregnant	34	79.7	6.7	57.2	92.5
cycles/pregnancy	34	2.0	0.3	1.5	2.9
Mare type					
maiden cycles/pregnancy	34	1.86	0.42	1.29	3.25
foaling cycles/pregnancy	34	1.96	0.32	1.50	3.00
barren cycles/pregnancy	34	2.36	0.54	1.64	4.00
slipped cycles/pregnancy	16	2.28	0.73	1.43	4.00
not bred cycles/pregnancy	18	2.41	1.41	1.00	6.50
% maiden pregnant	34	85	10	60	100
% foaling pregnant	34	79	8	55	93
% barren pregnant	34	78	10	60	96
% slipped pregnant	16	78	14	50	100
% not bred pregnant	18	81	24	29	100

Table 2. Fertility endpoints for a group of Thoroughbred stallions showing opportunity, breeding frequency, and seasonality values for a group of Thoroughbred stallions.

Opportunity to be bred	n	average	st. dev.	minimum	maximum
average day to first breeding	23	4-Apr	n/a	27-Mar	17-Apr
# doubles	34	8.6	5.0	0.0	23.0
cycles/mare	34	1.6	0.2	1.3	2.1
covers/cycle	34	1.1	0.0	1.0	1.2
# covers	34	195.1	78.9	97.0	356.0
Breeding Frequency					
% pregnant on days where only 1 mare was bred	34	50.1	9.3	30.4	70.2
% pregnant on days where 2 mares were bred	34	49.2	8.5	30.9	70.0
% pregnant on days where 3 mares were bred	33	49.2	10.2	25.0	70.2
% pregnant when 4 mares are bred	13	42.4	14.5	0.0	54.6
% pregnant when 5 mares are bred	1	66.7	n/a	66.7	66.7
Seasonality					
% pregnant for month February	34	50.3	14.6	11.1	73.3
% pregnant for month March	34	51.3	11.2	31.6	79.6
% pregnant for month April	34	50.7	8.2	29.7	66.7
% pregnant for month May	34	50.7	9.1	32.9	75.0
% pregnant for month June	33	45.9	15.9	12.5	75.0
% pregnant for month July	12	27.8	37.7	0.0	100.0

Figure 1 (stallion #1). This figure graphically represents an example of a highly fertile Thoroughbred stallion bred by natural cover. Note the cumulative pregnancy value continues to climb regardless of preceding breeding frequencies. There is no indication that a threshold breeding frequency, above which fertility declines, has been reached. While there are peaks and valleys in the pregnancy differential, they are probably related to non-stallion factors. This stallion would be expected to achieve a 90% seasonal pregnancy rate, requiring less than an average of 1.5 estrous cycles per pregnancy, in a book of a 100 mares bred by natural service.