As breeding season nears in North America, understanding the science behind what makes for a fertile stallion and mare is a helpful tool

Successful Reproduction

THE **SCIENCE** BEHIND **BREEDING**

By **AMANDA DUCKWORTH** | *Photos by* **ANNE M. EBERHARDT**

AS BREEDING SEASON approaches, farm managers actively work to make sure stallions and broodmares are at their best. Each mare that can get in foal on a single cover is a win for both sides of the equation. Understanding the science behind what makes for a fertile stallion or mare is a key part of Thoroughbred husbandry, and it remains a highly researched topic. The racetrack results of those successful breedings also make for interesting study.

While studies regularly occur in this area, it is also important to remember the basics. Healthy and fit horses have a much easier time being productive members of a breeding operation. The American Association of Equine Practitioners provides such a primer with its "Breeding Season Is Near" by Dr. Ben Espy.

"Mares should have a body condition score of 5 out of 10 to be effective reproducing animals," explained Espy. "Many studies have been performed that show that pregnant mares only require 1.4 to 1.6 times the calories of non-pregnant mares and only in the last 30 to 60 days of gestation. If mares become obese, foaling difficulties often occur.

"The reproductive needs of male horses often are neglected during this time of year. Stallion sperm production is maximized in the spring after they experience a quiescent winter period just like mares. Exposing mares to stallions has not been shown to speed a mare's return to cyclicity. Verify nutrition, deworming, and vaccination protocols with your veterinarian to ensure your stallions are in prime physical condition for the stressful breeding season that they are asked to endure."

Although there are obvious physical indicators that a stallion has covered a mare successfully, researchers recently reported findings on trying to determine in real time if that mating will result in a pregnancy. In March 2023, Reproduction published "Predicting the outcome of Thoroughbred stallion matings on the basis of dismount semen sample analyses."

"A capacity to predict the likelihood of pregnancy following natural matings would be of considerable benefit to the Thoroughbred horse breeding industry," explained researchers. "The purpose of this study was to determine whether the analysis of dismount semen samples from Thoroughbred stallions could be used to predict whether a given mating would result in a pregnancy."

For the study, researchers analyzed 143 matings of 141 broodmares by seven Thoroughbred stallions over a four-week period at an Australian stud. According to the researchers, the criteria of semen quality utilized in this analysis involved a preliminary assessment of the raw dismount sample in terms of semen volume, sperm number, and sperm movement characteristics using an iSperm® Equine portable device.

The researchers were able to

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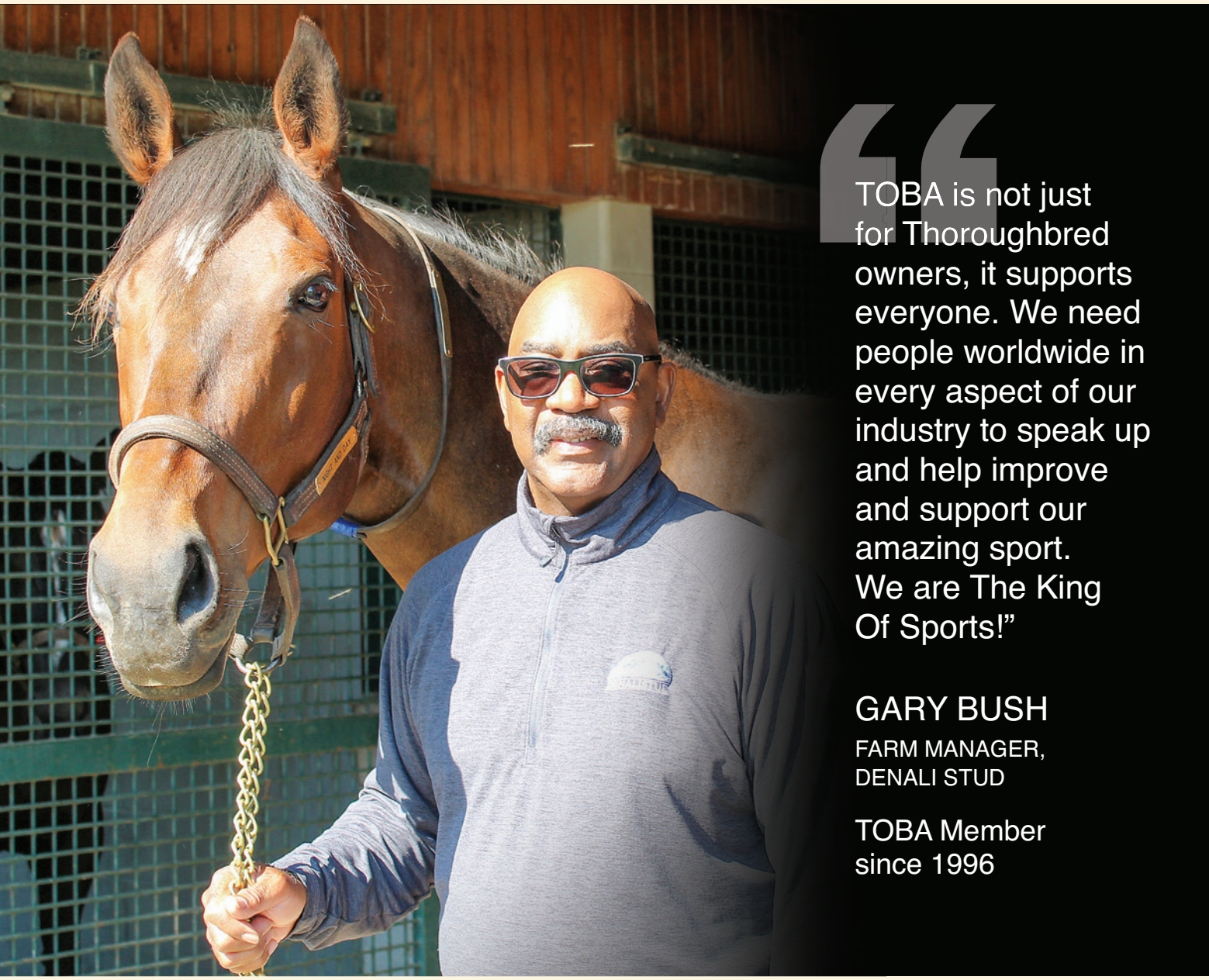

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successfully collect dismount samples from all of the matings monitored during the study period. They also gathered information on the ages of the stallions and broodmares, their historical breeding records, and mating frequency during the breeding season.

"The pregnancy rates observed during the study period varied between stallions, but not significantly," explained researchers. "However, the per stallion conception rate observed during the study period did correlate significantly with the rate for each stallion over the entire season, by the end of which, significant differences between stallions were evident with Stallion 4 being particularly fertile and Stallion 6 expressing significantly lower levels of fertility than the other stallions. Since there were significant differences in reproductive success between stallions over the breeding season, the discriminant analysis was repeated for each individual stallion."

A horse's fitness and health plays a role in being a productive member of a breeding operation

SKIP DICKSTEIN

Using the complete data set, researchers predicted the occurrence of pregnancy. Whether a mare was pregnant or not was then confirmed by ultrasound about 14 days after they were bred. Researchers found they were able to predict the outcome of a cover with an overall accuracy of 94.6%.

"The results of this analysis demonstrate that the quality of Thoroughbred

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stallion semen has a major impact on the likelihood that a natural cover will result in a pregnancy," concluded researchers. "Moreover, the quality of the entire ejaculate appears to be strongly correlated with the attributes of a dismount sample collected on withdrawal. These observations open up the possibility that rapid analysis of such samples will enable Thoroughbred breeders to determine whether a cover is likely to result in a pregnancy and thus whether the mare needs to be cross covered during the same estrous cycle to ensure a positive outcome.

"While female factors such as age and breeding history are absolutely acknowledged to be important determinants of pregnancy outcome in the Thoroughbred breeding industry, the results obtained in this study suggest that while the age of the mare may occasionally have an impact, the major determinant of a successful mating is semen quality."

Of course, the diversity of the breeding pool and the effects of selective breeding are always of note within the racing industry. The fact that the racing world is a global one can make matters more complex when it comes to trends in different areas.

In June 2022, Proceedings of the Royal Society, Biological Sciences published "Inbreeding depression and the probability of racing in the Thoroughbred horse."

A study in Australia analyzed dismount semen to see if it "could be used to predict whether a given mating would result in a pregnancy"

> **"IN CONTRAST TO RACING PERFORMANCE, INBREEDING HAD NO MEASURABLE EFFECT ON FOALING RATE OR GESTATION LENGTH IN AUSTRALIAN THOROUGHBRED HORSES."**
>
> —BMC GENETICS, APRIL 2020

"Small effective population sizes and active inbreeding can lead to inbreeding depression due to deleterious recessive mutations exposed in the homozygous state," explained researchers. "The Thoroughbred racehorse has low levels of population genetic diversity, but the effects of genomic inbreeding in the population are unknown.

"Purposeful inbreeding is common, with breeders attempting to leverage beneficial genetic variants by choosing mates with shared ancestors such that multiple ancestors may be duplicated in a five-generation pedigree. A recent trend of increasing inbreeding has been observed in the population. Inbreeding commonly leads to inbreeding depression, the reduced fitness of individuals due to deleterious recessive (or partially recessive) mutations exposed in the homozygous state. In livestock, for every 1% increase in (pedigree-estimated) inbreeding, there is an estimated 0.13% decrease in the mean selected trait value."

For the study, researchers quantified inbreeding based on runs of homozygosity (ROH) using 297 K SNP genotypes from 6,128 horses born in Europe and Australia, of which 13.2% were unraced. Race records up to the end of the 2020 racing season were retrieved for horses born prior to and including 2015, meaning the horses were therefore at least 5 years old.

Researchers concluded that a 10% increase in inbreeding (FROH) is associated with a 7% lower probability of ever racing. Additionally, a ROH-based genome-wide association study identified a haplotype on ECA14 which, in its homozygous state, is linked to a 32.1% lower predicted probability of ever racing, independent of FROH.

"The haplotype overlaps a candidate gene, EFNA5, that is highly expressed in cartilage tissue, which when damaged is one of the most common causes of catastrophic musculoskeletal injury in racehorses," explained researchers.

Mares under lights during foaling season

"Genomics-informed breeding aiming to reduce inbreeding depression and avoid damaging haplotype carrier matings will improve population health and racehorse welfare.

"Until now, the functional or clinical consequences of inbreeding in the Thoroughbred population were not known. Here we show, using SNP genotypes for a large cohort of Thoroughbred horses in two of the major global breeding regions, that inbreeding depression is a genome-wide phenomenon significantly impacting on the viability of a horse to ever race. Given the current pedigree structure of Thoroughbreds, avoiding breeding close relatives is challenging and the increasing levels of inbreeding in the population indicates that existing strategies to mitigate inbreeding may be inadequate."

While the prior study focused on Thoroughbreds born in Europe and Australia, the researchers also published "Inbreeding depression and durability in the North American Thoroughbred horse" in Animal Genetics in June 2023.

"The proportion of the genome containing runs of homozygosity (ROH) affects production traits in livestock populations," explained researchers. "In European and Australasian Thoroughbreds, inbreeding, quantified using ROH (FROH), is associated with the probability of ever racing. Here, we measured FROH using 333 K SNP genotypes from 768 Thoroughbred horses born in North America to evaluate the effect of inbreeding on racing traits in that region."

For the study, researchers looked at Thoroughbreds born in North America between 2008 and 2016. In the sample, 207 were males and 561 were females. Researchers obtained their race records, which spanned from Europe, Australasia, and North America for those 768 horses. They found 654 (85.2%) of them made at least one recorded race start, while 114 (14.8%) were unraced. Horses who did race made a median of 10 starts. Researchers then examined whether FROH is linked to a lower number of race starts among those horses who did make it to the races.

"Among North American horses, FROH was not associated with the probability of ever racing but was significantly associated with the number of race starts," concluded researchers. "Among raced horses, those with a 10%

higher FROH than the mean inbreeding coefficient were predicted to have 3.5 fewer race starts compared to horses with a mean inbreeding coefficient.

"Although commercial considerations and opportunity to race may have influenced this decline, here we show that inbreeding is also a significant contributing factor, presumably as a result of the cumulative effect of deleterious mutations across the whole genome. Therefore, efforts to mitigate inbreeding should be considered for breed improvement. Managing inbreeding in the population by determining genetic relatedness among potential mate choices and selecting less related animals for breeding, could improve the racing durability of horses in North America with long-term positive effects on population health and sustainability."

Inbreeding and how it can potentially impact outcomes has been examined in a variety of ways. In April 2020, BMC Genetics published "The effects of inbreeding on covering success, gestation length and foal sex ratio in Australian Thoroughbred horses."

"Horses produce only one foal from an 11-month gestation period, making the maintenance of high reproductive rates essential," explained researchers. "Genetic bottlenecks and inbreeding can increase the frequency of deleterious variants, resulting in reduced reproductive levels in a population. In this study we examined the influence of inbreeding levels on foaling rate, gestation length, and secondary sex ratio in Australian Thoroughbred mares. We also investigated the genetic change in these traits throughout the history of the breed."

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— DR. BEN ESPY

For the study, researchers examined phenotypic data from 27,262 breeding records of Thoroughbred mares provided by three Australian stud farms. They were able to estimate inbreeding by using the pedigree of each horse dating back to the foundation of the breed. Researchers found that while both gestation length and foaling rate were heritable, no measurable effect of inbreeding on either trait was found. That said, they did find that the genetic value for both traits had decreased within recent generations.

"In contrast to racing performance, inbreeding had no measurable effect on foaling rate or gestation length in Australian Thoroughbred horses," researchers concluded. "This could be because the level of inbreeding in the population examined is not high enough to show a discernible effect on reproductive traits. Populations that experience higher levels of inbreeding due to use of artificial reproductive technologies or extremely small population sizes may show a more pronounced reduction in natural foaling rate or gestation length.

"It is also possible that the intensive management techniques used in the Thoroughbred population masks any negative effects of inbreeding. The decrease in the genetic value of foaling rate is likely to be because horses with unfavorable genetic potential have not yet been selected out of the population. The change in genetic value of gestation length may be due to selective breeding favoring horses with shorter pregnancies. We also found that prioritizing the mating of older mares and avoiding out of season mating could lead to an increased breeding success."

Selecting the perfect stallion for a mare and vice versa is an art form in many ways. Being up to date on scientific research is a helpful tool managers can use in the quest for a successful pregnancy that hopefully results in a talented runner. BH