



Understanding exactly what makes a stallion fertile continues to be an area of study

Scientific Approach to Breeding

INFORMATION GROWING ON TOPICS SUCH
AS INFERTILITY, IMPACT OF INBREEDING

By AMANDA DUCKWORTH

AHEAD OF BREEDING season, farm managers everywhere are doing their best to have stallions and mares ready. Part of that effort is having both parties as close to optimal health as possible to ensure a successful year.

Fertility and genetic diversity remain major areas of research, and when it comes to stallions, these factors might become more important in determining a good candidate as understanding continues to grow.

The American Association of Equine Practitioners reminds anyone interested in standing stallions that a good working relationship with a licensed veterinarian is key to optimal breeding efficiency. Additionally, it recommends that to manage a stallion properly, a reproductive examination should be carried out by the veterinarian before a stallion enters a breeding program and periodically during his breeding career.

However, as its paper “Breeding Man-

agement Strategies and Semen Handling Techniques for Stallions—Case Scenarios” addresses, the highly managed breeding world does not necessarily match what occurs in nature.

“Stallions become sires based on three basic qualities: pedigree, performance record, and conformation,” explained the researchers. “Although stallions represent 50% of the breeding equation, the decision to retire a stallion to stud duty is made with little consideration to breeding capability or reproductive health. As such, the equine breeding industry abounds with stallions whose level of fertility is less than optimal.”

While most racehorses go on to adapt to stallion life with relative ease, there are plenty of examples of horses that were infertile or sub-fertile, making their stud careers short-lived or difficult.

Obviously, a stallion’s fertility is an important component to his success, but understanding exactly what makes a stallion fertile is a continued area of study and review. The issue was addressed in the October 2019 edition of *Reproduction* in the article, “What makes a fertile sperm? Unique molecular attributes of stallion fertility.”

The article notes that in Thoroughbred breeding there are challenges from the beginning of the process.

“Stallions experience lower per-cycle conception rates compared to other livestock species, largely because they are selected for breeding based on athletic prowess and not reproductive fitness,” explained researchers. “Mares are seasonal breeders, and pregnancies cannot be detected until 10-14 days post cover via transrectal ultrasonography. This means the detection of stallion fertility fluctuations is delayed by at least two weeks, which within the short breeding season employed by the Thoroughbred horse breeding industry, can prove quite costly.”

While more about stallion fertility is understood today than ever before, the need for further examination and

study could help future stallions reach higher fertility rates than their current counterparts.

“Deciphering the metabolic strategies employed by stallion spermatozoa has heralded the ability to use (reactive oxygen species, which are by-products of normal cell activity) production, antioxidant capacity, and DNA damage measurements as markers of sperm quality, predictors of fertility fluctuations, and indeed, diagnostic techniques for the investigation of stallion subfertility,” researchers said. “There has been increased focus on the use of proteomic tools in fertility biomarker discovery, with several sperm biomarker proteins identified in livestock species. Yet, stallion sperm proteomic studies thus far



One study suggests genomics-based approaches to identify genetic outcrosses can augment traditional methods of stallion selection for mares

have been largely descriptive in nature. Large-scale, quantitative analyses targeting the full proteome are needed in order to develop truly useful biomarker panels that are relevant across the breeding industry.

“Even less well studied are the roles of RNA species in stallion sperm function.

Future research must focus on elucidating the link between sperm RNA species and fertility, and in particular, whether non-coding RNAs participate in the spermatozoon’s acquisition of fertilizing capacity or contribute significantly to embryo development. Such studies will potentially provide additional means



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for predicting the breeding prospects of stallions, which will be crucially important for planning the breeding ‘careers,’ along with optimal welfare outcomes of these animals.”

The motility of a stallion’s sperm is an incredibly important factor when it comes to his ability to impregnate mares. This topic was examined in “Proteins involved in mitochondrial metabolic functions and fertilization predominate in stallions with better motility,” which was published in the September 2021 edition of *Journal of Proteomics*.

“Horses have been selected based on performance in sports and/or for morphological traits. This kind of selection has had little impact on fertility in comparison with most domestic species in which indirect selection for fertility has been conducted,” explained researchers. “As a consequence, large differences among stallions in terms of sperm quality are common.”

For the study, 10 different stallions of various breeds were used. Every stallion that took part had a proven track record of fertility, and semen was collected two to three times a week for the duration of the research.

“Even in stallions with sperm quality within normal reference ranges at ejaculation, subtle differences in sperm quality exist that in many cases lead to reduced time frames for conservation of the ejaculate and/or reduced fertility,” explained researchers. “The spermatozoon is a cell highly suitable for proteomics studies, and the use of this technique is allowing rapid advances in the understanding of sperm biology. The aim of the present study was to investigate differences among stallions of variable sperm quality (based on motility and sperm velocities), although all horses had sperm characteristics within normal ranges.”

Sperm motility; linear motility; and circular, straight-line and average velocities of the collected samples were measured using computer assisted sperm



Genetic diversity in the Thoroughbred continues to be a major area of research

analysis. A total of 903 proteins were identified in stallion spermatozoa, and of those, 24 were related to the percentage of total motility in the sample. Researchers found that in stallions showing better percentages of motility, and

lucida receptor complex,” concluded researchers. “The enrichment of this protein in samples with better percentages of total motility may offer a molecular explanation for the link between this parameter and fertility.”

Pairing the best with the best remains a cornerstone of breeding philosophies, but what that exactly means and a concern over dwindling sire lines are growing areas of interest. “Genomic inbreeding trends, influential sire lines and selection in the global Thoroughbred horse population” was published by *Scientific Reports* in 2020.

“The Thoroughbred horse is a highly valued domestic animal population under strong selection for athletic phenotypes,” explained researchers. “Here we present a high resolution genomics-based analysis of inbreeding in the population that may form the basis for evidence-based discussion amid concerns in the breeding industry over the increasing use of small numbers of popular sire lines, which may accelerate a loss of genetic diversity.”

Looking at a global sample of 10,118 Thoroughbreds, including 305 prominent stallions from the world’s major bloodstock regions, researchers found that there has been a significant decline in global genetic diversity over the course



IT IS, THEREFORE, HIGHLY LIKELY THAT OUR KNOWLEDGE OF THE REPRODUCTIVE ARCHITECTURE OF HORSES WILL GROW CONSIDERABLY OVER THE NEXT FEW YEARS.”

—FROM THE FEBRUARY 2021 ISSUE OF *ANIMALS* (BASEL)

circular and average velocity predominated mitochondrial proteins with roles in the citric acid cycle, pyruvate metabolism, and oxidative phosphorylation.

“Interestingly, in stallions with better percentages of total motility, sperm proteins were also enriched in proteins within the gene ontology terms, single fertilization, fertilization, and zona pel-

of the past 50 years. This conclusion was based upon pan-genomic SNP genotypes.

“Inbreeding is often a consequence of selection, which in managed animal populations tends to be driven by preferences for cultural, aesthetic, or economically advantageous phenotypes,” concluded researchers. “Using a composite selection signals approach, we show that centuries of selection for favorable athletic traits among Thoroughbreds acts on genes with functions in behavior, musculoskeletal conformation, and metabolism. As well as classical selective sweeps at core loci, polygenic adaptation for functional modalities in cardiovascular signaling, organismal growth and development, cellular stress and injury, metabolic pathways and neurotransmitters, and other ner-

vous system signaling has shaped the Thoroughbred athletic phenotype.

“Our results demonstrate that genomics-based approaches to identify genetic outcrosses will add valuable objectivity to augment traditional methods of stallion selection and that genomics-based methods will be beneficial to actively monitor the population to address the marked inbreeding trend.”

The desire to understand genetic diversity is a global one across breeds, and researchers recently examined the topic in “Genetic diversity and relationships among native Japanese horse breeds, the Japanese Thoroughbred and horses outside of Japan using genome-wide (single nucleotide polymorphisms) data.” The study was published in the October 2019 edition of *Animal Genetics*.

For the study, researchers compared genetic diversity among 32 international horse breeds previously evaluated by the Equine Genetic Diversity Consortium, the eight Japanese native breeds, and Japanese Thoroughbreds.

“Eight horse breeds—Hokkaido, Kiso, Misaki, Noma, Taishu, Tokara, Miyako and Yonaguni—are native to Japan,” explained researchers. “Although Japanese native breeds are believed to have originated from ancient Mongolian horses imported from the Korean Peninsula, the phylogenetic relationships among these breeds are not well elucidated. Researchers found that the proportion of polymorphic loci and expected heterozygosity showed that the native Japanese breeds, with the exception of the Hokkaido, have

Continued on page 138

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WHEN IT COMES TO HORSE HEALTH & SAFETY, DON'T HIT THE EASY BUTTON

Many horse owners want to reduce the cost of treatment by reaching for a “compounded” version of altrenogest (a progestin used in veterinary medicine to suppress or synchronize estrus in horses) in long-acting injectable formulations. BUT AT WHAT COST TO YOUR HORSE?

A compounder simply mixes up a drug preparation and sells it *without any required testing for purity and concentration*. This has been illustrated many times by horses DYING from compounded medications that weren't tested before being sold. *Compounded products require no proof of efficacy*, so you have no proof the product is even altrenogest or is safe.

When you use only FDA approved altrenogest products such as Altren® (altrenogest) Oral Solution manufactured by Aurora Pharma-



ceutical, the veterinarian and the horseman know the *ingredients have been tested for purity* and the final product has been *tested for purity and stability*. NO EXCEPTIONS. Also, before any drug formula is approved by the FDA, it must pass rigorous research trials that prove it is safe and works for its intended purpose.

So, the question every equine enthusiast must ask is *whether convenience is more important than the peace of mind that comes from using the approved and tested product in your expensive mare?* The answer should always be NO. Your equine partner will thank you.

—Content provided by Aurora Pharmaceutical, Northfield, Minn. <https://aurorapharmaceutical.com>

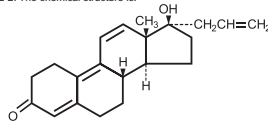
Altren® (altrenogest)

SOLUTION 0.22% (2.2 mg/mL)

CAUTION: Federal law restricts this drug to use by or on the order of a licensed veterinarian.

DESCRIPTION:

Altren® (altrenogest) Solution 0.22% contains the active synthetic progestin, altrenogest. The chemical name is 17 α -allyl-17 β -hydroxyestra-4,9,11-trien-3-one. The CAS Registry Number is 850-52-2. The chemical structure is:



Each mL of Altren® (altrenogest) Solution 0.22% contains 2.2 mg of altrenogest in an oil solution.

ACTIONS:

Altren® (altrenogest) Solution 0.22% produces a progestational effect in mares.

INDICATIONS:

Altren® (altrenogest) Solution 0.22% is indicated to suppress estrus in mares. Suppression of estrus allows for a predictable occurrence of estrus following drug withdrawal. This facilitates the attainment of regular cyclicity during the transition from winter anestrus to the physiological breeding season. Suppression of estrus will also facilitate management of prolonged estrus conditions. Suppression of estrus may be used to facilitate scheduled breeding during the physiological breeding season.

CONTRAINDICATIONS:

Altren® (altrenogest) Solution 0.22% is contraindicated for use in mares having a previous or current history of uterine inflammation (i.e., acute, subacute, or chronic endometritis). Natural or synthetic gestagen therapy may exacerbate existing low-grade or “smoldering” uterine inflammation into a fulminating uterine infection in some instances.

PRECAUTIONS:

Various synthetic progestins, including altrenogest, when administered to rats during the embryonic stage of pregnancy at doses manyfold greater than the recommended equine dose caused fetal anomalies, specifically masculinization of the female genitalia.

DOSAGE AND DIRECTIONS:

While wearing protective gloves, remove shipping cap and seal; replace with enclosed plastic dispensing cap. Remove cover from bottle dispensing tip and connect luer lock syringe (without needle). Draw out appropriate volume of Altren® solution. (Note: Do not remove syringe while bottle is inverted as spillage may result.) Detach syringe and administer solution orally at the rate of 1 mL per 110 pounds of body weight (0.044 mg/kg) once daily for 15 consecutive days. Administer solution directly on the base of the mare's tongue or on the mare's usual grain ration. Replace cover on bottle dispensing tip to prevent leakage. Excessive use of a syringe may cause the syringe to stick; therefore, replace syringe as necessary.

DOSAGE CHART:

Approximate Weight in Pounds	Dose in mL
770	7
880	8
990	9
1100	10
1210	11
1320	12

WHICH MARES WILL RESPOND TO ALTREN® (altrenogest) SOLUTION 0.22%:

Extensive clinical trials have demonstrated that estrus will be suppressed in approximately 95% of the mares within three days; however, the post-treatment response depended on the level of ovarian activity when treatment was initiated. Estrus in mares exhibiting regular estrus cycles during the breeding season will be suppressed during treatment; these mares return to estrus four to five days following treatment and continue to cycle normally. Mares in winter anestrus with small follicles continued in anestrus and failed to exhibit normal estrus following withdrawal.

Response in mares in the transition phase between winter anestrus and the summer breeding season depended on the degree of follicular activity. Mares with inactive ovaries and small follicles failed to respond with normal cycles post-treatment, whereas a higher proportion of mares with ovarian follicles 20 mm or greater in diameter exhibited normal estrus cycles post-treatment. Altrenogest Solution 0.22% was very effective for suppressing the prolonged estrus behavior frequently observed in mares during the transition period (February, March and April). In addition, a high proportion of these mares responded with regular estrus cycles post-treatment.

SPECIFIC USES FOR ALTREN® (altrenogest) SOLUTION 0.22%:

SUPPRESSION OF ESTRUS TO:

- Facilitate attainment of regular cycles during the transition period from winter anestrus to the physiological breeding season. To facilitate attainment of regular cycles during the transition phase, mares should be examined to determine the degree of ovarian activity. Estrus in mares with inactive ovaries (no follicles greater than 20 mm in diameter) will be suppressed but these mares may not begin regular cycles following treatment. However, mares with active ovaries (follicles greater than 20 mm in diameter) frequently respond with regular post-treatment estrus cycles.
- Facilitate management of the mare exhibiting prolonged estrus during the transition period. Estrus will be suppressed in mares exhibiting prolonged behavioral estrus either early or late during the transition period. Again, the post-treatment response depends on the level of ovarian activity. The mares with greater ovarian activity initiate regular cycles and conceive sooner than the inactive mares. Altren® (altrenogest) Solution 0.22% may be administered early in the transition period to suppress estrus in mares with inactive ovaries to aid in the management of these mares or to mares later in the transition period with active ovaries to prepare and schedule the mare for breeding.

- Permit scheduled breeding of mares during the physiological breeding season. To permit scheduled breeding, mares which are regularly cycling or which have active ovarian function should be given Altren® (altrenogest) Solution 0.22% daily for 15 consecutive days beginning 20 days before the date of the planned estrus. Ovulation will occur 5 to 7 days following the onset of estrus as expected for non-treated mares. Breeding should follow usual procedures for mares in estrus. Mares may be regulated and scheduled either individually or in groups.

ADDITIONAL INFORMATION:

A 3-year well controlled reproductive safety study was conducted in 27 pregnant mares, and compared with 24 untreated control mares. Treated mares received 2 mL altrenogest solution 0.22%/110 lb body weight (2x dosage recommended for estrus suppression) from day 20 to day 325 of gestation. This study provided the following data:

- In filly offspring (all ages) of treated mares, clitoral size was increased.
- Filly offspring from treated mares had shorter interval from Feb. 1 to first ovulation than fillies from their untreated mare counterparts.
- There were no significant differences in reproductive performance between treated and untreated animals (mares & their respective offspring) measuring the following parameters:
 - interval from Feb. 1 to first ovulation, in mares only.
 - mean interovulatory interval from first to second cycle and second to third cycle, mares only.
 - follicle size, mares only.
 - at 50 days gestation, pregnancy rate in treated mares was 81.8% (8/11) and untreated mares was 100% (4/4).
 - after 3 cycles, 11/12 treated mares were pregnant (91.7%) and 4/4 untreated mares were pregnant (100%).
 - call offspring of treated and control mares reached puberty at approximately the same age (82 & 84 weeks respectively).
 - stallion offspring from treated and control mares showed no differences in seminal volume, spermatozoal concentration, spermatozoal motility, and total sperm per ejaculate.
 - stallion offspring from treated and control mares showed no difference in sexual behavior.
 - testicular characteristics (scrotal width, testis weight, parenchymal weight, epididymal weight and height, testicular height, width & length) were the same between stallion offspring of treated and control mares.

REFERENCES:

Shenckel, C.F., E.L. Squires, and R.K. Shideler, 1989. Safety of Altrenogest in Pregnant Mares and on Health and Development of Offspring. Eq. Vet. Sci. (9), No. 2: 69-72.
Squires, E.L., R.K. Shideler, and A.O. McKinnon, 1989. Reproductive Performance of Offspring from Mares Administered Altrenogest During Gestation. Eq. Vet. Sci. (9), No. 2: 73-76.

WARNING:

For oral use in horses only. Keep this and all other medications out of the reach of children. Do not use in horses intended for human consumption.

HUMAN WARNINGS:

Skin contact must be avoided as Altren® (altrenogest) Solution 0.22% is readily absorbed through unbroken skin. Protective gloves must be worn by all persons handling this product. Pregnant women or women who suspect they are pregnant should not handle Altren® (altrenogest) Solution 0.22%. Women of child bearing age should exercise extreme caution when handling this product. Accidental absorption could lead to a disruption of the menstrual cycle or prolongation of pregnancy. Direct contact with the skin should therefore be avoided. Accidental spillage on the skin should be washed off immediately with soap and water.

INFORMATION FOR HANDLERS:

WARNING: Altren® (altrenogest) Solution 0.22% is readily absorbed by the skin. Skin contact must be avoided; protective gloves must be worn when handling this product.

Effects of Overexposure

There has been no human use of this specific product. The information contained in this section is extrapolated from data available on other products of the same pharmacological class that have been used in humans. Effects anticipated are due to the progestational activity of altrenogest.

Acute effects after a single exposure are possible; however, continued daily exposure has the potential for more untoward effects such as disruption of the menstrual cycle, uterine or abdominal cramping, increased or decreased uterine bleeding, prolongation of pregnancy and headaches. The oil base may also cause complications if swallowed.

In addition, the list of people who should not handle this product (see below) is based upon the known effects of progestins used in humans on a chronic basis.

PEOPLE WHO SHOULD NOT HANDLE THIS PRODUCT:

- Women who are or suspect they are pregnant.
- Anyone with thrombophlebitis or thromboembolic disorders or with a history of these events.
- Anyone with cerebral-vascular or coronary-artery disease.
- Women with known or suspected carcinoma of the breast.
- People with known or suspected estrogen-dependent neoplasia.
- Women with undiagnosed vaginal bleeding.
- People with benign or malignant tumors which developed during the use of oral contraceptives or other estrogen-containing products.
- Anyone with liver dysfunction or disease.

Accidental Exposure

Altrenogest is readily absorbed from contact with the skin. In addition, this oil based product can penetrate porous gloves. Altrenogest should not penetrate intact rubber or impervious gloves; however, if there is leakage (i.e., pinhole, spillage, etc.), the contaminated area covered by such occlusive materials may have increased absorption. The following measures are recommended in case of accidental exposure.

Skin Exposure: Wash immediately with soap and water.

Eye Exposure: Immediately flush with plenty of water for 15 minutes. Get medical attention.

If Swallowed: Do not induce vomiting. Altren® (altrenogest) Solution 0.22% contains an oil. Call a physician. Vomiting should be supervised by a physician because of possible pulmonary damage via aspiration of the oil base. If possible, bring the container and labeling to the physician.

Store at or below 25° C (77° F). Reclose tightly.

HOW SUPPLIED:

Altren® (altrenogest) Solution 0.22% (2.2 mg/mL). Each mL contains 2.2 mg altrenogest in an oil solution. Available in 1000 mL and 150 mL plastic bottles.

Manufactured by:
Aurora Pharmaceutical, Inc.
Northfield, Minnesota 55057



Approved by FDA under ANADA # 200-620

04/2019



FDA-approved Altren® (altrenogest) Solution 0.22% is indicated to suppress estrus in mares. Altren is the only oral progestin available in a 150 mL single horse dosing package

FDA APPROVED – quickly and safely reduces the moodiness and temperament changes often associated with estrus and keeps your mare out of heat for all her major events

PROVEN PERFORMANCE – contains the same active ingredient and dosing regimen as Regu-Mate®

ECONOMICAL PACKAGING – unique, 150 mL individualized treatment package keeps product cost down and assures effective treatment



The Only FDA-Approved Vented Draw-Off Cap



Managing Estrus to Fit Your Schedule

Altren® (altrenogest) Solution 0.22% is contraindicated for use in mares with a previous or current history of uterine inflammation. Talk to your veterinarian about proper use and safe handling of Altren. Avoid skin contact and always wear protective gloves when administering. Pregnant women, or women who suspect they are pregnant, should not handle Altren. Refer to the package insert by visiting www.aurorapharmaceutical.com for complete product information.

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MANUFACTURED IN THE USA A4000069 10/2019

For more information on Altren® consult your veterinarian or equine health care professional

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Breeding

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relatively low diversity compared to the other breeds sampled.

“The Japanese Thoroughbreds were distinct from the native breeds, and although they maintain similar overall diversity as Thoroughbreds from outside Japan, they also show evidence of uniqueness relative to the other Thoroughbred samples. This is the first study to place the eight native Japanese breeds and Japanese Thoroughbred in context with an international sample of diverse breeds.”

Understanding how horse genomics can improve overall breeding selection practices for Thoroughbreds is a frontier in equine research. The progress in this area was reviewed in “Impaired Reproductive Function in Equines: From Genetics to Genomics,” which was published in the February 2021 edition of *Animals* (Basel).

“Horse genomics is currently undergoing an exponential expansion, not least due to the adaptation of new genomic methodologies to the species, the existence of a new, accurate reference genome, and the exponential increase in the number of equines which have been genotyped,” said researchers. “It is, therefore, highly likely that our knowledge of the reproductive architecture of horses will grow considerably over the next few years.

“However, large-scale datasets of reproductive phenotypes are still scarce in horses, probably due to the lack of availability of reliable reproductive phenotypes (particularly in mares). Therefore, the development of new phenotypes to measure reproductive fitness more objectively and their systematic use by breeder associations are essential to allow a more in-depth study of the reproductive function in horses.”

The amount of work going into this field of study continues to grow, as discussed in “Generation of a Biobank From Two Adult Thoroughbred Stallions for the Functional Annotation



Thoroughbreds aren't the only breed in which genetic diversity is being examined; researchers recently studied this topic in Japanese breeds such as the Misaki

of Animal Genomes Initiative,” which was published in March 2021 by *Frontiers in Genetics*.

“Following the successful creation of a biobank from two adult Thoroughbred mares, this study aimed to recapitulate sample collection in two adult Thoroughbred stallions as part of the Functional Annotation of the Animal Genome initiative,” explained researchers. “Both stallions underwent thorough physical, lameness, neurologic, and ophthalmic (including electroretinography) examinations prior to humane euthanasia.”

Researchers said such an approach is not their first choice but that the study results will provide future benefits for horses.

One of the Thoroughbred stallions used for the study was a 3-year-old who had been in training before suffering a career-ending injury while the other was 4 years old and had not been in training. Epididymal sperm were recovered from both stallions immediately postmortem and cryopreserved while under the guidance of a board-certified veterinary anatomic pathologist; 102 representative tissue samples were collected from both horses.

“DNA from each was whole-genome sequenced and genotyped using the GGP Equine 70K SNP array,” explained researchers. “The genomic resources and banked biological samples from these animals augment the existing resource available to the equine genomics community. Importantly we may now improve the resolution of tissue-specific gene regulation as affected by sex, as well as add sex-specific tissues and gametes.

“This undertaking was no small feat and required the sacrifice of two additional animals. However, the benefit to future equine genomic studies is immense. Although this study concludes the tissue collection arm of the project, the work to annotate the equine genome continues in earnest. This addition to the biobank expands the list of tissues available to the equine research community.”

The Thoroughbreds with the best pedigrees that live up to those expectations on the track will always continue to be the most in-demand stallions upon retirement. However, as science continues to look within, recognizing what will make a stallion successful from the perspective of breeding soundness and fertility is becoming more understood by the day. **BH**