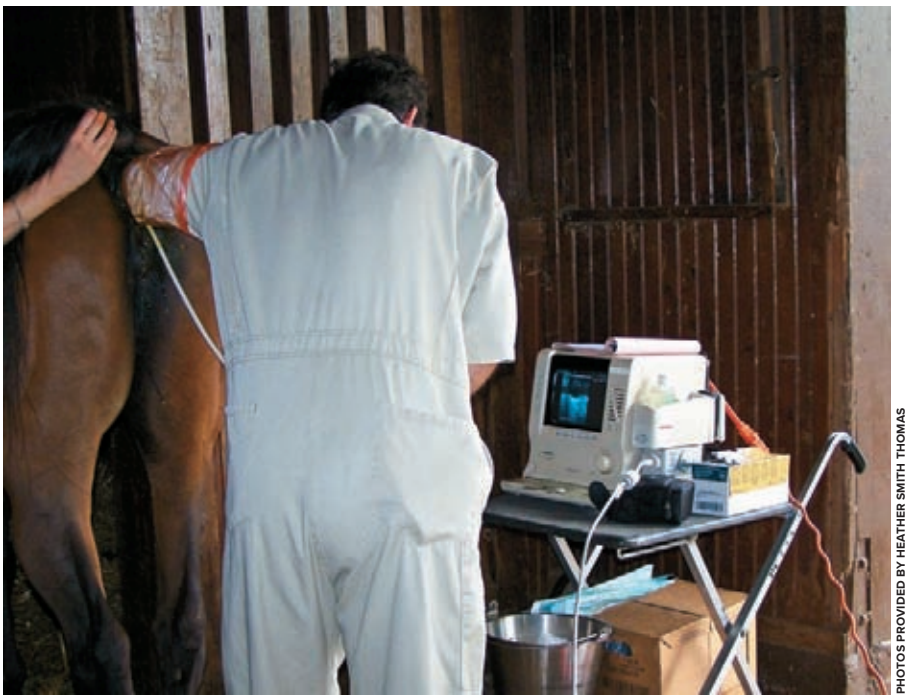
Reproductive Veterinary Services

Optimizing success in equine reproduction

BY HEATHER SMITH THOMAS



PHOTOS PROVIDED BY HEATHER SMITH THOMAS

Scanning a mare for pregnancy

BREEDING HORSES IS OFTEN challenging, with the goal of getting every mare safely and efficiently in foal. There can be frustrations along the way, and it sometimes takes a dedicated team of mare/stallion managers and reproductive veterinarians to get the job done to maximize pregnancy rates. Many farm veterinarians do excellent jobs, sometimes assisted by reproductive specialists at equine hospitals or universities when working with a problem mare or stallion. Some of the routine methods of dealing with reproductive problems have improved over the past several decades, and there are also new technologies that have helped fine-tune these methods and some new advances in enhancing mare or stallion fertility.

Dr. Maria Schnobrich (a board-certified theriogenologist at the LeBlanc Reproduction Center at Rood & Riddle Equine Hospital) works with her repro group at Rood & Riddle and several universities on studies looking at ways to improve fertility. She says continued collaboration between clinical work and research-based reproductive studies has helped optimize success in equine reproduction.

"Through combined efforts of farm managers, horse owners, and veterinarians (and an open mind to new research), we continue to make advances," she said. "Several new procedures and management practices have been explored recently, but the most important factors are those we already know."

MAKE SURE THE MARE IS SUITABLE FOR BREEDING

Thoroughbred mares entering a breeding shed are often required to have a "suitable for mating" examination by a veterinarian.

"This will determine whether a mare's reproductive tract is healthy, and if the mare is ready for breeding (appropriate stage of her estrous cycle) and will tolerate a stallion breeding by natural cover," Schnobrich said. "We use a combination of physical examination, trans-rectal palpation, and ultrasound of the reproductive tract and vaginoscopy, as well as obtaining a clean endometrial culture (free of bacterial growth) and normal endometrial cytology, prior to breeding. We check for any abnormalities that could reduce the likelihood of a successful mating or might be potentially harmful to the stallion.

"Abnormalities would include evidence of sexually transmissible diseases (coital exanthema or EHV-3), large amounts of fluid in the uterus, evidence of infection or urine pooling, or conformational abnormalities that could be a problem during mating," she continued. "We also obtain a culture and cytology before breeding, to ensure there is no evidence of clinical infection or excessive inflammation."

Any problems need to be dealt with and resolved before the mare is bred.

"We might not be able to have 100% evidence there are no issues, but at this

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MESSAGE FROM THE GRAYSON-JOCKEY CLUB RESEARCH FOUNDATION

PLACENTITIS, 20 YEARS LATER... WHAT HAVE WE LEARNED?

BY DR. MARGO MACPHERSON

IN 1999 THE LATE DR. MICHELLE LEBLANC was awarded a grant by the Grayson-Jockey Club Research Foundation to investigate factors contributing to the disease process of ascending, equine placentitis.

Using ambitious methodology, Le Blanc's research team established a working model of induced placentitis using a transcervically-placed inoculum of clinically isolated *Steptococcus equi* subspecies *zooepidemicus*. While some would argue the model does not realistically represent the clinical condition, the information gained from this model has positively improved the outcome of many mares affected with placentitis. The question is: what exactly have we learned about equine placentitis?

Placentitis in mares is most commonly caused by bacteria ascending through the vagina. The pathogen most commonly implicated in equine placentitis is *Streptococcus equi* subspecies *zooepidemicus*. Led by LeBlanc, workers from the University of Florida conducted several coordinated studies that provided formative information regarding the pathophysiology of this disease.

Important information gained from these studies revealed that after bacteria migrated through the mare's cervix, infection and inflammation of the placenta followed. Uterine contractions occurred earlier, and with greater strength, in infected mares, thus causing early delivery of premature foals. Some, but not all, aborted fetuses had bacterial infections. Some foals were delivered prematurely and survived. From these, and studies in other species, it is thought that some foals from infected mares are prompted to mature more quickly, in utero, due to the indirect production and secretion of cortisol.

Ultimately, treatment strategies have been directed at resolving bacterial infection, reducing inflammation and maintaining pregnancy long enough so that fetal maturation occurs and the foal survives.

Several studies that have been funded by the GJCRF have investigated diagnostic and treatment protocols for mares with placentitis to improve foal survival. Physical and ultrasonographic examination findings in mares "at risk" for placentitis remain the mainstay for initial diagnosis of disease. However, clinical findings often lag well behind the disease process and treatment. Ongoing studies supported by the GJCRF are investigating metabolomics, or the use of small metabolites found in cells, biofluids or tissues, for earlier diagnosis of placentitis.

Therapeutic agents that are used clinically have also been tested for placental passage and improved foal viability in normal pregnant mares and mares affected with placentitis. Commonly used drugs such as penicillin and trimethoprim sulfamethoxazole have been shown to achieve effective concentrations against *S. zooepidemicus* in allantoic fluid of mares with induced placentitis. Similarly, gentamicin was detectable at concentrations effective to treat *Escherichia coli* and *Klebsiella pneumoniae* (also implicated in placentitis) in mare allantoic fluid.

Based on drug concentrations, these antimicrobial drugs may be appropriate for treating placentitis. Ceftiofur sodium and ceftiofur crystalline free acid were not detected in fetal fluids or placental and fetal tissues, but did have a pharmacologic profile in pregnant mares that was similar to non-pregnant animals. Thus, cetioufur-based drugs are not good antimicrobial drugs for treating mares with placentitis but can be useful for treating other disease (i.e. respiratory) in pregnant mares.

Anti-inflammatory drugs are an important part of placentitis treatment protocols. Pentoxifylline was detected in allantoic fluid of experimentally-infected mares, but flunixin meglumine was not. Firocoxib, a potent anti-inflammatory drug has recently been shown to achieve concentrations in fetal fluids as well as fetal and placental tissues after administration to normal mares. GJCRF studies are ongoing to determine whether firocoxib, physiologically, impacts the placentitis disease process. These studies hope to provide evidence to support the use of firocoxib in mares with placentitis.

Delayed delivery and improved foal survival are important end points when treating mares with placentitis. Long term administration of TMS, pentoxifylline, and progestins (altrenogest; Regumate™) to mares with induced placentitis resulted in the majority of mares (10/12; 83%) delivering live foals.

Untreated, infected mares aborted or delivered non-viable foals. This treatment regimen has been broadly adopted in clinical practice with varying results. More recently, a group of seven mares with placentitis were administered TMS, firocoxib, and altrenogest. All seven mares delivered live foals. While results from this small study do not suggest a "silver bullet" treatment for mares with placentitis, they do encourage further investigation of this drug regimen in a large population of mares.

In summary, equine placentitis is a challenge both diagnostically and therapeutically. Yet, salvaging a pregnancy can be enormously rewarding. Ongoing efforts by several investigators are focusing on methods for earlier diagnosis of disease, allowing for more rapid initiation of effective treatments and delivery of more viable foals. BH

Dr. Margo MacPherson is a reproduction professor at the University of Florida and a Grayson-Jockey Club Research Foundation-funded researcher.

point in time, these are the most routine, thorough, and practical things that we can do," said Schnobrich. "Sending a mare that is not suitable uses valuable covers for the stallion and might (in the case of transmissible diseases) pose risk for the breeding population."

It is also important to have mares in good body condition—not overly fat or thin. Being too fat is worse than being underweight. Mares that are somewhat thin but gaining weight are more likely to cycle properly and become pregnant.

"It is well known in other domestic species such as sheep, that if they are 'flushed'—on an increasing plane of nutrition before breeding—they are more fertile and have higher pregnancy rates. We try to have these mares in proper body condition and gaining weight going into January," she said.

Being current on appropriate vaccinations is also important, following the American Association of Equine Practioners guidelines for pregnant mares.

"In the fall it might also be helpful to vaccinate with the new equine vaccine for *Leptospira pomona* to help prevent pregnancy loss," she said. "As far as we know, there have been no abortions reported from leptospirosis in mares that have been vaccinated." Vaccinations against equine herpes virus (EHV-1) should be administered at three, five, seven, and nine months of gestation to aid in prevention of equine abortion.

"We also try to address any systemic health issues, lameness, chronic pain, etc. Mares should be screened for overall health and checked for equine endocrine disorders such as equine metabolic syndrome or pituitary pars intermedia dysfunction (or Cushings) sometime in the fall/winter before the breeding season and make sure any pathology is well regulated for at least 60 days coming into the breeding season," Schnobrich said. "You want everything ready to go, for optimal chances for success."

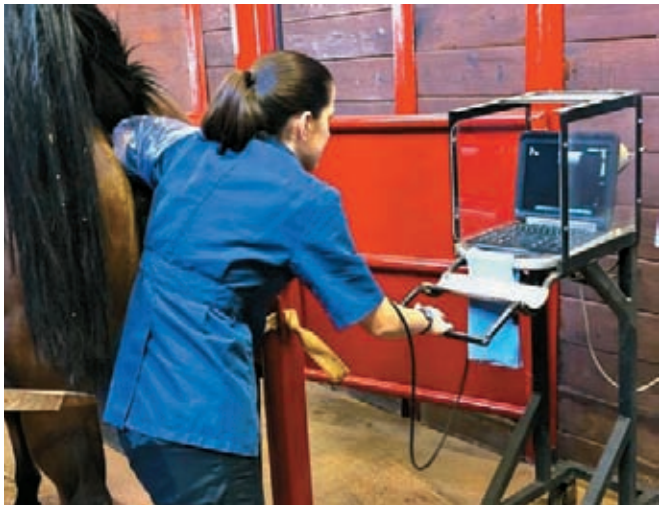
At the end of breeding season, it is just as important to make sure mares are in good reproductive health if they are not pregnant.

"Any reproductive surgeries that need to be done should be taken care of before the next breeding season," she said. "These might include cervical repairs, perineal body reconstruction (after foaling injuries), and urethral extensions to prevent urine pooling. There are many chronically infected mares because they continually collect urine in the uterus, and are not diagnosed or addressed.

"You also want a clean endometrial culture and cytology, with no evidence of problems," Schnobrich continued. "It is detrimental to the mare's reproductive tract to go through the whole winter with an infection. It is imperative to clear it up at the end of the breeding season, to protect and optimize uterine health for the next year."

Uterine cysts and oviduct pathology are usually addressed in early January to allow time for resolution of any inflammation caused by these procedures but not so far away from breeding season that a mare might develop new cysts or reocclude an oviduct.

"New advances in treatment for oviduct pathologies include hysteroscopic flushing or application of prostaglandins on the oviduct or its opening into the uterus," she said. "If we suspect a plugged oviduct (which occurs in a very small subset of mares), we might apply prostaglandins to the oviduct or flush it, and this sometimes helps."



Performing a rectal ultrasound

Because the Thoroughbred breeding season starts in early to mid-February it is important to make sure mares are under lights at the appropriate time and that lighting is adequate so mares can go through the transition from anestrus to cycling early.

"There is a mask that a mare can wear, to allow administration of artificial lighting when they are outdoors rather than confined in a stall," Schnobrich said. "This is something to consider for mares that do better moving around, such as arthritic individuals or mares that don't do well in confinement."

Manipulation of breeding season can be accomplished by providing 14-16 hours of light daily, starting at least eight to 10 weeks prior to the breeding season.

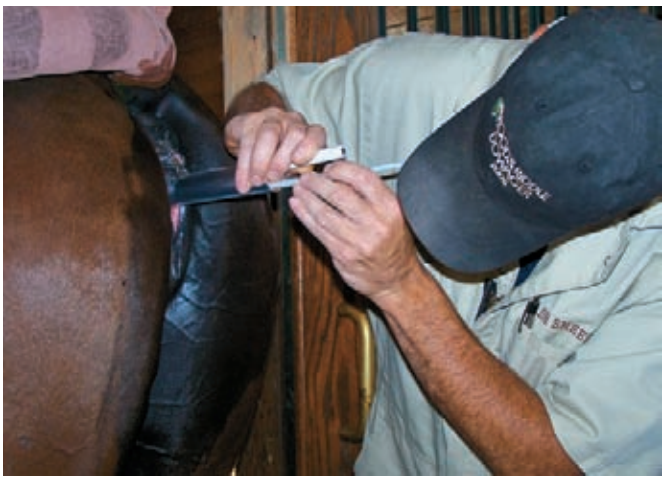
"This regimen is typically begun Dec. 1. A combination of natural or ambient light can be utilized as well as artificial light from the Equilume mask or in a paddock or stall," she said.

LAB ASSAY TO TEST FOR BIOFILM

"If the mare has a persistent infection, there is a new assay that we can use for testing to see what the best treatment option might be, for the microbe that was isolated from the mare's uterus," Schnobrich said. "It is thought that some of the mares that have persistent infections (that won't clear) might have bacteria living in a dormant state or within a biofilm that offers the bacteria some protection. These bacteria have increased resistance to antibiotics, sometimes requiring much higher concentrations of antibiotics to kill them. We need ways to disrupt the biofilm or use a different mechanism to kill them.

"We've been working with Dr. Ryan Ferris who developed an assay that we can use for testing, through his work on equine biofilm while he was at Colorado State University," she continued.

"His work demonstrated that biofilm is generated in the mare's uterus with infection of certain bacteria. For those of us who are trying to resolve these infections, we use biofilm disrupters like hydrogen peroxide, Tricide, N-acetylcysteine, or DMSO (dimethyl sulfoxide). Now we can test to see which of these treatments would be most efficacious against a particular biofilm. We can take a culture from the mare and determine whether she has a bacterial infection and if those bacteria produce a biofilm, we can run this test to determine which type of medication would work best to treat that specific biofilm. The cultured bacteria forms a biofilm, and then we test it with these agents to see which one gives the most reduction in biofilm and bacteria."



Obtaining a culture sample

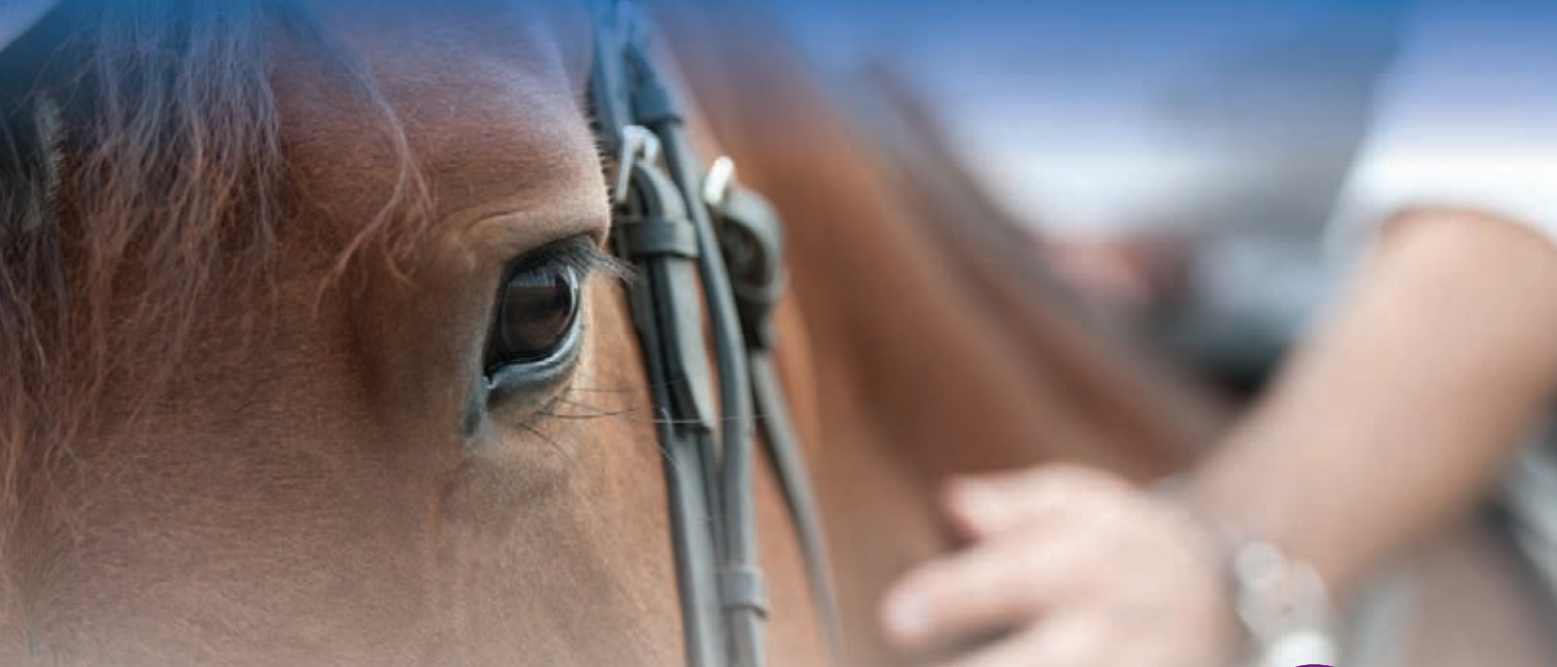
This might be one more way to treat mares that are difficult to get pregnant because of persistent infection and could lead to a more rapid resolution of the infection.

"This should help us provide a more targeted treatment for these mares because it helps us know which agent will be best to use," she explained.

MANAGING OVULATION

Some of the things that negatively impact a mare in terms of reproductive ability are her age and the time elapsed between breeding and ovulation.

"We must ensure timely ovulation relative to breeding. Though factors such as age and stallion subfertility might be difficult to overcome, ensuring that your mare ovulates soon after breeding will increase likelihood of establishing pregnancy," Schnobrich said. "In Thoroughbred breeding, the longer the time span from the time she is bred to the time she ovulates, the lower her per-cycle pregnancy rate. It is very important that your mare is man-



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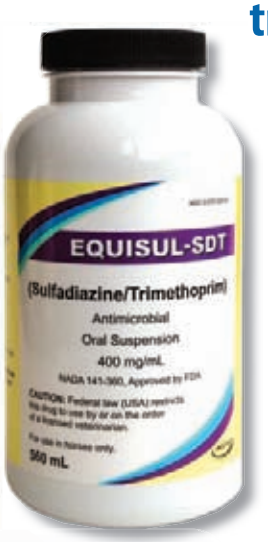

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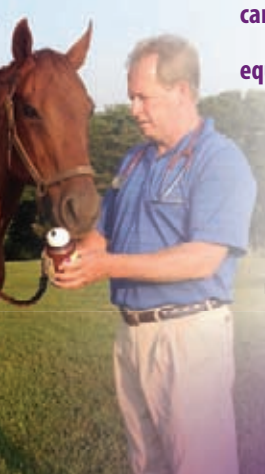

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THE STALLION

One thing we can do to try to optimize pregnancy rates is look at a stallion's previous fertility and what percentage of his mares became pregnant.

"If you ask the stud farm about how a certain stallion has done in the past, it is important to ask two questions," says Dr. Maria Schnobrich (a board-certified theriogenologist at the LeBlanc Reproduction Center at Rood & Riddle Equine Hospital in Lexington).

"One is what was his per-cycle pregnancy rate (settling a mare on her first cycle) the previous year, and this is the most important question," she said. "The per-cycle rate should be above 60% and is a more sensitive indication of a stallion's fertility. Per-cycle pregnancy rate measures how efficient a stallion is at establishing a pregnancy. The other question is, what was his end-of-season pregnancy rate; of the mares bred, how many did he get pregnant that season."

According to Dr. Stephanie Walbornn, (another theriogenologist at Rood & Riddle) a stallion can have a very low per-cycle pregnancy rate but still have a high end-of-season pregnancy rate, given enough time to rebreed the mares.

"This is because the seasonal pregnancy rate does not take into account how many times the same mare is bred, over consecutive cycles," Walbornn said. "Therefore, the seasonal pregnancy rate can be an overestimation of the true number of pregnancies a stallion can obtain. Increasing the number of times a stallion breeds the same mare (over several cycles) increases the amount of physical work for that stallion and the costs for both the stallion and mare owners. Ideally, one cover per mare would result in a pregnancy."

In the Thoroughbred industry it is important to have foals born early, so breeders want mares bred and settled as soon as possible.

"You don't want to breed to a stallion that takes multiple tries. If he is batting 40% or lower per cycle, there might be additional management techniques needed, to try to improve those percentages," Walbornn said.

"With natural cover we can perform reinforced breeding, and some studies have shown this can help stallions that have low per-cycle pregnancy rates. After the stallion covers a mare, the dismount sample is taken, extended, and inseminated into the mare's uterus. With some stallions, this technique is beneficial."

Schnobrich says most stud farms utilize management procedures to help these stallions, such as limited book size, limiting covers to just two per day—spread very far apart—and reinforcement breeding. As a mare owner or manager, it is important to ask about these things and find out whether this is being done.

"Often your own veterinarian can be there at the breeding to do the reinforcement breeding, or the stud farm might be able to provide a veterinarian, or they might be able to do it themselves, but this is something to think about, to optimize the mare's chances that she could settle to that breeding," Schnobrich said.

Walbornn says some stallions might need an increased number of covers per day in order to improve their pregnancy rate.

"These stallions are known as 'accumulators.' They accumulate sperm within their accessory sex glands (ampullae), which can prevent the ejaculated sperm from reaching the urethra and being deposited into the mare's uterus," she said. "Mares might not become pregnant because only a small quantity of abnormal stagnant sperm is being deposited into their uterus.

"Management techniques for these stallions include increasing the number of covers per day, administering ecbolic agents such as oxytocin or prostaglandin F2 alpha prior to breeding, or trans-rectal massage of the ampullae prior to breeding. Regardless of the reason for a low per-cycle or seasonal pregnancy rate, it is important to try to maximize your chance of pregnancy by optimizing fertility in both the stallion and the mare."

"In general, horses are very fertile animals, and when using live cover we assume that for each mating there should be about a 65-70% chance the mare will conceive to that cover," Schnobrich said.

In summary, evaluation of the whole horse (mare or stallion) is important, because reproductive success occurs when the horse is in peak health. Working with experienced farm managers, well-rounded veterinarians, and reproductive specialists who can provide extra assistance if needed, helps horse owners achieve optimal results. The reproductive teams at referral hospitals and specialized repro facilities work hard to stay involved with current research, and collaborate with veterinarians to bring the newest advances to the field for improvement of the horse.

—By Heather Smith Thomas

aged so that she ovulates within 24-48 hours from breeding."

The egg only lives a certain length of time but the bigger issue is semen viability and longevity. If the mare ovulates more than 48 hours later (after breeding), some stallions' semen won't last long enough and there might not be enough sperm present to facilitate conception.

"The egg might be ready, but the sperm might not survive that long. The stallion might have poor longevity; the sperm might not be able to maintain motility that long, or the stallion might be breeding so many mares that his sperm numbers are too low," she said. "When the mare finally ovulates, after two days there might be no viable sperm left."

It is very important from a mare management standpoint to determine (and plan breeding relative to) when she will ovulate.

"With most Thoroughbred mares we recommend giving an ovulation-inducing agent the day before breeding," Schnobrich said. "Mare owners should make sure their veterinarian is able to optimize the time of ovulation, and give the mare this drug 24 hours before breeding, so she will ovulate in that ideal time frame (12 to 24 hours after breeding)." This will give the best chance for conception.

"Additionally, administration of cloprostenol (Estrumate) four to six hours after breeding has shown some evidence it might help a mare ovulate sooner or more reliably, as well as assist with uterine clearance post-mating," she said.

POST-BREEDING MANAGEMENT

Your veterinarian should evaluate the mare for evidence of endometritis (inflammation of the endometrium—the inner lining of the uterus) and manage treatment accordingly.

"This might be accomplished with post-breeding lavages, or use of a mucolytic-like acetylcysteine (which helps thin and loosen mucus), or ecbolic drugs like oxytocin to aid uterine clearance (stimulating contractions of the uterus)," she said. "We want to get the uterus as healthy as possible after breeding (get rid of any debris—seminal fluids, dead sperm, bacteria, etc. that was introduced by breeding) before the embryo comes into the uterus from the oviduct, about six days after ovulation.

"It takes that long for the embryo to make its way through the oviduct and into the uterus. This gives us a window of time to get the uterine environment clean so it will be a healthy place for the embryo to attach. It's a multi-step process. We try to get the mare to ovulate on time, and now we need to optimize the uterine environment so the embryo will survive."

Diet and nutrition are also important in terms of being proactive in preventing inflammation.

"Some mares are on a very high carbohydrate diet, and we've found that adding things such as diet change and addition of omega-3 fatty acids might be helpful for modulating inflammation," Schnobrich said. "This can be helpful for broodmares that are prone to becoming inflamed during breeding season, and there is also evidence of beneficial effects in stallions as well. There are many good feed products that are high in omega-3, but it is good to evaluate the diet and look at the whole picture."

MONITORING PREGNANCY

"The next step is monitoring the early pregnancy, making sure you don't have twins, and no evidence of hormonal abnormalities (low progesterone) or persistent inflammation. Then we continue to monitor the health of the pregnancy to see if there is anything else that needs to be done," Schnobrich said. "There might be concerns later in gestation (after 100 days) about ascending placentitis or other pathologies, and the mare might benefit from treatment. The overall health, vaccination, and comfort of the mare are important for producing a healthy foal. There are many things we need to keep track of."

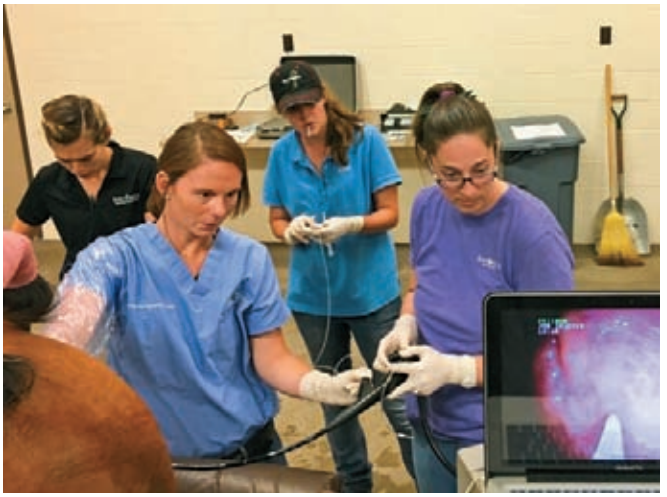
Once the mare is pregnant, you want to keep her that way and ensure a healthy gestation and a healthy foal.

FLUSHING A PLUGGED OVIDUCT (OVIDUCTAL HYDROTUBATION)

"This is something that can sometimes help a mare become pregnant if other efforts fail. There are many different pathologies that can cause a mare to not become pregnant, but in this instance we often are not sure exactly what is causing the infertility, and we might utilize this flush if the mare is unable to establish a pregnancy," she said.

"The mare usually has a history of producing pregnancies and then just stops. She might have a year when you can't get her pregnant at all. At the 14-day pregnancy check there is no embryo. It can be a maiden mare that will not become pregnant despite appropriate breeding management, or a mare that's had pregnancies in the past."

The flushing procedure involves use of a sterile solution,



Performing an oviduct flush

flushed from the uterus into the oviduct, toward the ovary. This is a standing procedure with the mare sedated.

"The idea is to mechanically alter the oviduct and restore function. This can sometimes help a mare become pregnant if all other efforts have failed and all other pathologies have been ruled out," Schnobrich said.

"The mares that we would try this with are mares who have no known genetic issues and cannot produce a pregnancy despite appropriate breeding management; there is no infection or any other pathology that could explain the failure to conceive, after multiple sires have been tried."

The mare's oviduct is unique compared to other domestic animals.

"There is a small sphincter that only allows the embryo to pass through and not unfertilized eggs. We don't know whether the oviduct in this area is prone to closing off because of debris or some other problem, but the flush basically cleans it out and seems to restore fertility in some cases," said Schnobrich.

There are some other treatments that have been used in the past with good success to remedy this problem, but the flush is easier and does not require surgery.

"We do the flush using hysteroscopy with a little camera on the endoscope so we can identify and locate the opening of the oviduct, and flush fluid through it," she said. "This procedure seems to work on a select group of mares and was developed by a Japanese veterinarian, Dr. Yuji Inoue, who has published research on its efficacy. If we do appropriate case selection (mares that we suspect might have this problem, with the correct history and everything else is normal), about 60-70% of those mares become pregnant within two covers. We did a study on this a few years ago and found that this procedure can be very effective, given the right case selection.

"Recent work by Dr. Stephanie Walbornn has shown similar efficacy between oviduct flushing and prostaglandin (PGE2) gel applied to the oviduct. Both procedures are available. There might be further advances in using a PGE1 (just applying that to the oviduct opening) but further research needs to be done."

The oviduct flush or application of the gel is one more thing a person could try if a mare fails to become pregnant.

SPECIALIZED REPRODUCTIVE FACILITIES

There are times a certain mare (or stallion) might be sent to a specialist or referral facility where specific problems could be more fully evaluated and addressed. There are several referral clinics/hospitals that have specialists who do this. Your veterinarian might be able to do what is needed, or might want to work with a specialist to get some answers or further treatment options. Sometimes it takes a team effort, working together.

"We do breeding soundness exams and assist equine reproduction with traditional and routine breeding work, but if a mare or stallion needs more advanced assistance, we are able to do some of these procedures. We also have horses referred to us for more advanced diagnostics," said Schnobrich.

"Usually we get these at the end of the breeding season when the mare has not become pregnant and the resident veterinarian has run out of options. Probably the most helpful thing we can do is a hysteroscopic exam where we look into the uterus. We often do this with mares that have had persistent infections.

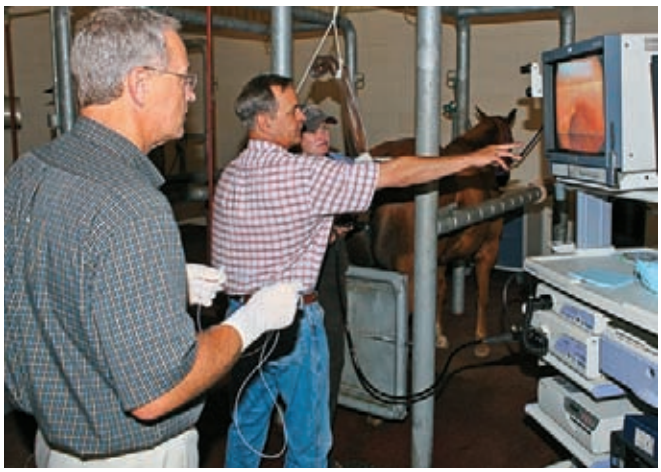
We generally find that they have adhesions, foreign bodies, or scarring in the uterus," she said.

"In my opinion this type of exam is an important tool because there are mares that are chronically 'dirty,' yet you can't determine why. A mare owner might spend thousands of dollars trying to figure out and resolve the infection and never get her cleared up."

Some of these problems won't show up with ultrasound.

"When we go in with the scope, we might find there is serious scarring that cannot be detected by ultrasound. We've seen mares with uterine horns closed off or huge scars or adhesions that would prevent pregnancy from developing normally, but you wouldn't know it from any other test except the hysteroscopic exam. We feel that this exam and a biopsy are helpful tools for the owner to have realistic expectations about the mare, and we could potentially diagnose something that was previously undiagnosed," she said.

Some of these problems can be addressed and resolved, and some might not be resolvable. "Either way we need to know. With some mares, owners and veterinarians have tried for a number of years to get them in foal, unsuccessfully, and then we check them and find the uterus is too scarred to carry a pregnancy. Not knowing that condition, you could waste a lot of money trying to get them pregnant."



Reviewing a mare's hysteroscopy

There are many reproductive experts available, so the farm veterinarian might decide to send a mare to a referral facility for further diagnostics and help. BH

Heather Smith Thomas is a freelance writer based in Idaho.