Two Much

BY AMANDA DUCKWORTH

AT THE CLOSE OF 2019, Catch Ya Later Bro and Twin Turbo made headlines for their exploits. Victories in maiden claimers rarely lead to such things, but in this case, the story was rather unusual. The two are twin brothers, as the Storm Bird mare Andrea Gail gave birth to both of them May 8, 2017. Catch Ya Later Bro won at Tampa Bay Downs in late December, matching the success of his brother, Twin Turbo, who had broken his maiden at Hawthorne in October.

It was a wonderful but highly unlikely result for the duo. Broodmares are not designed to carry twins, and when they do, it is rare they both end up healthy, much less in the winner's circle.

A broodmare's health, as well as the viability of her pregnancy, is significantly compromised by carrying twins to term. The idea that twins are undesirable is well known, but the science behind when it occurs in Thoroughbreds and how to proceed remains important. Especially when one considers the fact Thoroughbreds are far more likely to end up having a twin pregnancy than their Quarter Horse counterparts.

The paper "Success rates of various techniques for reduction of twin pregnancy in mares," which was published in the *Journal of the American Veterinary Medical Association* in July 2014 explains it thusly: "Twinning is an important cause of pregnancy loss in mares and is often associated with economic loss. Abortion in late gestation and the birth of twins often involve dystocia and trauma to the reproductive tract and are associated with poor rebreeding potential.

> Broodmares are not designed to carry twins

"Twinning appears to have a high degree of repeatability and heritability. The rate of occurrence can vary according to breed and may be directly proportional to the fertility of the stallion. The prevalence of multiple ovulations is thought to be highest in Thoroughbreds, Warmblood breeds, and Irish draft horses."

Because a mare's uterus is not designed to carry simultaneous pregnancies, it is estimated that only about 1% of twin pregnancies results in two healthy foals. Although identical equine twins—which result from a fertilized egg splitting in two—can occur, it is much more common for twins to happen as the result of the stallion's sperm fertilizing two eggs released from two different follicles due to the mare double-ovulating.

A mare can ovulate twice at around the same time or several days apart, and sperm can live inside the mare's reproductive tract for several days. Because Thoroughbreds are more prone to ovulate multiple times compared with other breeds, they are more likely to conceive twins. According to the American Association of Equine Practitioners, the percentage of twin pregnancies in Thoroughbreds is estimated to be as high as 25-35% of all conceptions while it only occurs in Quarter Horses around 5-10% of the time.

The fact double ovulation can occur several days apart also helps explain why twins can be missed during routine early pregnancy checks.

"Multiple pregnancies largely derive from multiple ovulations, most commonly double ovulations, hence, double ovulations are of significant concern to breeders/veterinarians," explains the study "Asynchronous ovulation in mares: seasonal variations in frequency," which was published in the *Veterinary Record*

SV PHOTOGRAPHY

Catch Ya Later Bro, one of a set of twins, breaking his maiden at Tampa Bay Downs

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MESSAGE FROM THE GRAYSON-JOCKEY CLUB RESEARCH FOUNDATION

Q&A WITH DR. SOPHIE H. BOGERS

Grayson-Jockey Club Research Foundation

Dr. Sophie H. Bogers, of Virginia Tech's Marion duPont Scott Equine Medical Center, has conducted research focused on using stem cells to treat osteoarthritis in horses with specific aims of optimizing the anti-inflammatory properties of equine bone marrow-derived mesenchymal stem cells. Bogers currently serves as an assistant professor at Virginia Tech and was a 2015 recipient of the Klein Family Award from the Grayson-Jockey Club Research Foundation.

The award is a competitive program intended to promote development of promising investigators by providing a one-year salary supplement of $15,000. The award is named in memory of renowned horsewoman Elaine Klein and her late husband, Bertram. It is funded by the Klein family. What follows is a brief Q&A with Bogers.

COURTESY DR. SOPHIE H. BOGERS

Dr. Sophie H. Bogers

What first sparked your intellectual curiosity to explore this area of equine research? Have you studied this area of equine research before?

As an intern in Kentucky in 2010, I was exposed to the expanding industry of regenerative medicine and saw the detrimental effects musculoskeletal disease could have on horses' careers. Some benefits of regenerative therapies were being realized, but I wanted to understand their therapeutic mechanisms and how to enhance their potential. Being primarily interested in surgery and lameness, I wanted to ask questions about stem cells in the context of orthopedic disease. Of all the orthopedic diseases that face our horses, osteoarthritis is perhaps the most common and challenging as once the cycle of chronic inflammation and tissue breakdown begins it is impossible to stop or repair the damage. Dr. Jennifer Barrett mentored me to put these interests together in the context of my PhD, which explored how the physical and biochemical environment could affect the therapeutic potential of equine bone-marrow derived stem cells. I had no background in stem cell or molecular biology, so it was a learning curve. But with excellent mentorship and determination I was able to couple my existing knowledge of veterinary medicine with stem cell biology to do clinically relevant research.

What was the most significant finding from this research? What, if anything, surprised you about your findings?

We learned it can be therapeutically beneficial to alter the environment of stem cells before they enter the disease environment. For inflammatory conditions, such as the low-grade inflammation in osteoarthritis, this is important because stem cells need to be triggered into an anti-inflammatory state. It's not only the culture conditions that affect the stem cells, but also what horse they came from and the level of inflammation they encounter in the horse. We have known for a while that stem cells are sensitive to all sorts of donor-related and environment-related factors; it's just rewarding to find specific relationships and use them to a therapeutic advantage.

What did you learn about the research process through your project?

I knew the PhD would take a good degree of perseverance, especially when things don't go to plan. What I didn't fully appreciate was how important the relationships I built would be on the ease of doing my experiments. When it came to long hours of live-horse experiments, it was amazing the number of people who put their hands up to volunteer. It showed me the research process is about involving and motivating a range of people who just want to help horses and horse health. I also learned that it's important to personally embrace and be involved with all aspects of the research process because the continuity contributes to improved quality and professional development.

How will this research improve equine health and welfare?

Knowing how stem cells respond to their environment for therapeutic applications is the initial step to producing effective stem cell therapies. The ultimate goal is to produce an anti-inflammatory and regenerative stem cell therapy for osteoarthritis. The more basic research we do the more we will understand how to reach our ultimate goal. It is an extremely exciting goal to work toward because, if achieved, it will alter the outlook for so many of our horses with osteoarthritis, which is currently a progressive, degenerative disease with no cure.

Has this research led to additional projects?

That's the excellent thing about research...one question answered generates many more questions. We are keen to use similar concepts in other species and for different diseases. We will continue developing the techniques we used during these experiments.

What is next?

I am starting a faculty position at Virginia Tech. It will be exciting to continue to practice equine surgery as well as contribute to equine stem cell and orthopedic research. My mentors at Virginia Tech have been amazing, and it will be great to continue to work with them and also build other relationships outside the university. BH

in March 2015. "Double ovulations may be synchronous or asynchronous. Asynchrony of up to 96 hours may result in two embryonic vesicles of very different sizes, which are challenging to detect at early ultrasonic pregnancy detection."

Embryos usually do not appear on an ultrasound until 11 days after ovulation, so a second egg that was fertilized several days after the first might not appear on a routine Day 14 exam.

This is an issue for multiple reasons, including the fact that after about 16 days, embryos stop moving and attach at the base of one of the mare's uterine horns. If there are two embryos, it is estimated that they attach to the same horn about 70% of the time. The larger embryo almost always crushes the smaller, but the two embryos can potentially fuse, making the elimination of one unlikely, which in turn would mean both potential foals would be lost.

COURTESY KING RANCH INSTITUTE

Dr. Benjamin Espy, the author of 'Equine Reproduction from Conception to Birth' for the AAEP

The study recorded the results from 506 cycles in double-ovulating mares that had not received ovulation induction agents and found that 65.8% of double ovulations occurred more than eight hours apart and 28.5% of double ovulations occurred more than 24 hours apart. Additionally, more asynchronous double ovulations occurred in seasonal transition periods, meaning at the beginning and end of the breeding season.

"This study demonstrates that asynchronous double ovulation is common and emphasizes the importance of closely monitoring mares, particularly at the extremes of the breeding season, for double ovulation up to or at 96 hours post-initial ovulation in order to minimize the chances of missing multiple pregnancy," the authors concluded.

The ultrasound has greatly changed the twin landscape in Thoroughbred mares, and it is a key tool when keeping tabs on a mare's pregnancy.

"If you consistently check mares at Day 14, at some point you will miss a younger twin that is 12 or 13 days old and too small to be visualized," explained Dr. Benjamin Espy in his paper "Equine Reproduction from Conception to Birth" for the AAEP. "Regardless of what day of gestation you check for twins, it is much easier to reduce a twin before they become fixed at Day 17 of gestation.

"Reducing twins is also called 'crushing' a twin. This has only been possible since the advent of ultrasound. Before this time, veterinarians and owners often didn't know until the mare aborted. The smaller twin is usually crushed. The mare is typically examined 48-72 hours after the procedure to confirm that the remaining embryo survived."

It is important to eliminate the smaller embryo in a detected twin pregnancy for safety, and this method is effective more than 90% of the time. Odds are high that Mother Nature is going to get rid of the second embryo anyway, but it might come at a time that impacts the health of the mare and/or the viable foal.

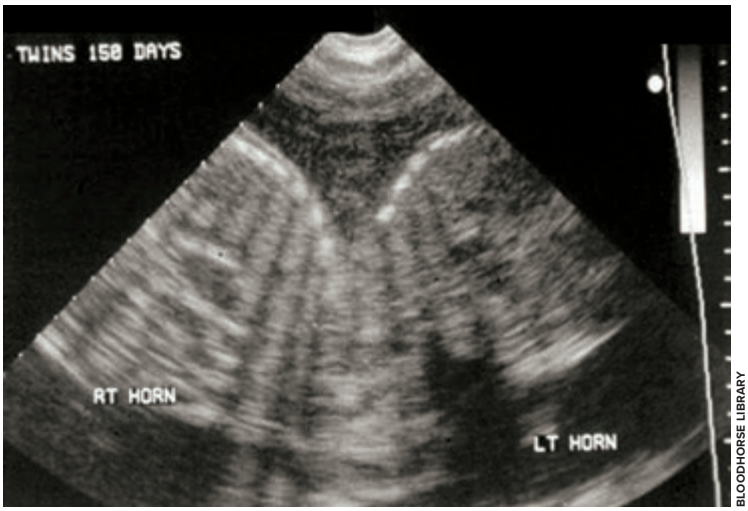
According to the AAEP, in up to 95% of mares with twin embryos, one or both embryos are resorbed or aborted during the first 60 days. Twins that do survive past that mark will often be spontaneously aborted at six to eight months. This is obviously a major health concern for the mare. Twins that do make it are more likely to be born prematurely, which can lead to serious medical problems and even death.

Manually reduced multiple pregnancies have proved to be effective and safe for the mare although it is important to note that the success rate diminishes past Day 25 because of the size of the embryos.

During the 2010 AAEP convention, Dr. Pete Sheerin reviewed

a study he had conducted on manual twin reduction using the medical records of mares that underwent twin reduction at Rood & Riddle Equine Hospital.

"This study was performed to determine whether pregnancy loss after manual twin reduction was affected by a veterinarian performing the procedure, drug treatment, or mare age," he explained. "Live foal rate of mares that underwent a twin reduction was lower (80.3%) than that of control mares (86.7%). There was no difference in live foal rates in mares that underwent a twin reduction on days 13–16 of gestation and mares 17–20 days of gestation."



An ultrasound, at 150 days, shows twins are being carried

While the veterinarian performing the procedure affected live foal rates, the differences were not related to experience, as some were specifically given more challenging cases. Unsurprisingly, older mares and those not treated with medicine did have higher rates of failure.

"Twinning mares greater than 15 years of age had a lower foaling rate (66.2%) than younger mares (83.2%)," Sheerin said. "Control mares older than 15 years of age also had lower foaling rates compared with young control mares (77.6% versus 89%). Mares treated with flunixin meglumine and progesterone had higher foaling rates than mares receiving other treatment or no treatment (82% versus 77%).

In 2012 the *Veterinary Journal* published a study entitled "Manual reduction of multiple embryos in the mare: the effect on subsequent pregnancy outcome," which concluded it was a safe and worthwhile procedure.

"To determine whether manually reduced multiple pregnancies (MPs) are at a greater risk of pregnancy loss than single pregnancy (SP) in mares, and to examine if a difference exists in the timing of pregnancy loss between manually reduced MPs and SPs, 1,916 Thoroughbred mares were ultrasonically monitored every two days during oestrus to confirm ovulation, and up to Day 40 post-ovulation to confirm pregnancy," explained the study.

"Ultimate pregnancy outcome was ascertained from the *General Thoroughbred Stud Book* and classified as live foal, early abortion (Days 40-150), slipped foal (Days 150-term), and barren. Significantly more SPs failed (17.23%) than manually reduced MPs (13.41%).

"Both SPs and MPs were at greatest risk of being lost as early abortions (72.16% and 61.67%, respectively) compared to slipped foals (27.84% and 38.33%). There was no significant difference in the time of greatest risk of pregnancy loss between manually reduced MPs and SPs. It was concluded that owners and veterinarians can be assured that manual reduction of MPs does not increase the risk of pregnancy loss above those associated with SPs."

If a mare is discovered to be carrying twins later in her pregnancy, there are options, but they come with more complications than manually reducing the pregnancy in its early days. One available method is for veterinarians to try to dislodge one of the embryos, but because it contains more fluid at this stage, that fluid can affect the other embryo's development.

Another possibility until about the 60- day mark is transvaginal ultrasound-guided aspiration (TUA), but it is encouraged that this procedure is also done sooner rather than later.

In "Effects of mares' age and day of gestation on efficacy of transvaginal ultrasound-guided twin reduction," which *Tierarztliche Praxis* published in June 2018, 88 mares pregnant with twins were evaluated. TUA of the yolk sac or allantoic fluid was performed between day 30 and 62 of gestation, and the mares were aged 3-22.

"Four weeks after TUA, 67% of the cases resulted in a viable singleton pregnancy," the study found. "Five to seven days after TUA treatment, the success rate was 74%. The gestational period did not affect the outcome, irrespective of the age of the mare. In contrast, success rates decreased with increasing age of the mares.

"TUA was found to be an effective procedure for reduction of twin pregnancies performed at days 30-62 of gestation. Success rates for singleton pregnancies were high for young mares (84%) and middle-aged mares treated before day 36 of pregnancy (74%). Duration of pregnancy at the time of TUA did not have a major impact on the outcome. Nevertheless, the procedure should optimally be performed around days 32-35 of pregnancy to allow for the possibility of natural reduction before treatment and rebreeding in case of a total pregnancy loss after TUA."

Options remain available past the two-month mark but become increasingly complicated and less likely to succeed. Early twin detection and reduction significantly increase the chances of a viable single foal birth and help protect the health and well-being of the mare. BH

Amanda Duckworth is a freelance writer based in Lexington.