Reproduction

BY LES SELLNOW

Milne Lecture

Dickson Varner, DVM, MS, Dipl. ACT (a specialist in reproduction), is a self-described "renegade" when it comes to presenting papers at veterinary gatherings. He is known for his irreverent humor, poetry, and clever turn of phrase. That being said, Varner is also a leading researcher in equine reproduction and has helped propel Texas A&M University into a leadership role in that field.

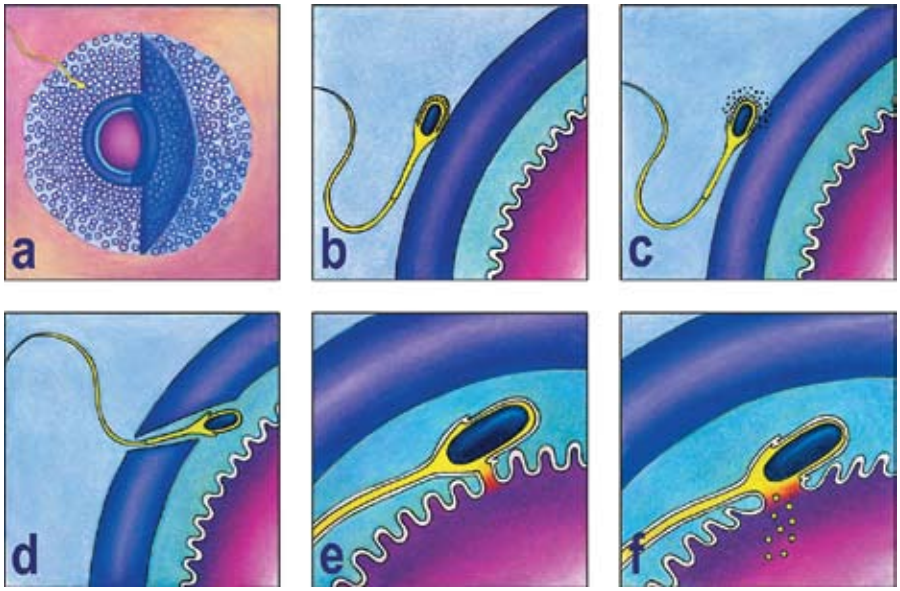
He was chosen to present the Milne State-of-the-Art Lecture at the convention. The lecture and award were named in honor of Frank Milne, the late and longtime editor of the annual AAEP Convention *Proceedings*. The lecture is designed to offer in-depth information on a particular subject.

Varner lectured on the topic: "From a Sperm's Eye View—Revisiting our Perception of This Intriguing Cell."

Varner said the lecture was not a compilation of his work, but a presentation of the work from a great many scientists through the years. As if to prove that point, his paper published in the *Proceedings* contained more than 1,000 references.

Dickson's ditty It was truly an in-depth description of the sperm cell, but it also contained Varner's trademark brand of humor. He said that program chairman and 2008 AAEP President Eleanor Green, DVM, Dipl. ACVIM, ABVP, and 2007 AAEP President Doug Corey, DVM, had frequently expressed concern about what bent his humor might take and worried that he might get out of line. He jokingly offered to don a dog shock collar and said they could have the remote control button and activate it whenever he went over the edge in the humor department.

Among other things, Varner is a cowboy. He grew up working with his parents in a Wild West show, complete with rough stock and trick animal acts, and he went on from there to be a rodeo contestant before joining the academic path. Thus, it came as no real surprise when he announced that an animated cowboy would help him with his message during the Milne Lecture. He introduced "Dr. Dick," an out-of-work cowboy, who would ride a sperm through a series of slides depicting the cell's travels from the time it is formed until it ends up entering the female oocyte and establishing a pregnancy.



COURTESY DR. DICKSON VARNER/AAEP

Illustration of how a spermatozoon fertilizes an egg. a) The spermatozoon navigates through the egg's protective barrier and b) adheres to the inner layer. c) The sperm cell's protective covering begins to disintegrate. d) The sperm gains entry into the egg. e) The protective covering dissolves, allowing the sperm cell's DNA to merge with that of the egg (f).

Everyone who knows Varner also knew he would do something unique when he arrived at the halfway point of his lecture and it was time for a break. He didn't let them down. He harked back to the 1998 Milne Lecture presented by O.J. Ginther, VMD, PhD, of the University of Wisconsin, and did him one better. During his break, Ginther, who was presenting a paper on equine pregnancy, picked up a guitar and sang a ditty titled "Mare is Four-Letter Word." It was a hit.

Varner brought to the stage two professional musicians from Texas, Aaron Watson and Dan McBride, as backup, and he sang a song titled "Stud is a Four-Letter Word." He also whipped out his trademark harmonica to play along, and the large crowd responded with a standing ovation.

Origin of the sperm With the help of "Dr. Dick," whose spermatozoal ride got rather hectic and scary at times—especially during ejaculation—Varner described the origin of the sperm within the testis "the sperm factory."

The process is called spermatogenesis. "Spermatogenesis," he wrote in the paper he presented, "is an extremely complex process that involves germ proliferation, germ cell differentiation, and, paradoxically, programmed germ cell death (termed apoptosis). This lengthy process, which is 57 days in the stallion, is controlled by a vast array of cell-signaling messengers acting through endocrine, paracrine (when target cell is close to signal-releasing cell), juxtacrine (cells must be in direct contact), and autocrine (via secretion of a substance) pathways."

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The seminiferous tubules within the testis are where the spermatozoa are manufactured, Varner said. If the tubules from two stallion testes were stretched out end to end, he said, they would be 50 football fields in length. As such, these tubules are capable of producing 60,000 to 70,000 spermatozoa per second. If one were to line up the number of sperm produced by a stallion in a normal lifetime, Varner said, they would stretch to the moon and back more than three times.

The main storage area for the sperm within the testis is the epididymis, which if stretched out would be about three-fourths of a football field in length. Each epididymis is capable of storing 40 to 45 billion sperm. During their time in the epididymis, the spermatozoa undergo a maturation process that is required for interaction with the mare's reproductive tract and establishment of fertilizing potential.

Prolonged storage of the sperm within the epididymis is not necessarily a good thing and problems can arise, Varner said, in the form of bent tails, detached heads, distal droplets (abnormalities on the sperm flagella, or tails), and decreased motility. Normally, spermatozoa enter the epididymis at a constant rate in a reproductively normal stallion, with about 5 billion arriving each day.

The stay within the epididymis is from eight to 11 days. After their stay in the epididymis, sperm are either ejaculated or spontaneously ejected into the urethra and expelled. This latter mechanism ensures that viable sperm are available for fertilization at the time of mating or artificial insemination.

A Sperm's Tale Varner described an individual spermatozoa as being shaped like a tadpole, with a head and a flagellum. Stallion spermatozoa are about 70 to 80 microns in length. They are only about half as long as mouse spermatozoa.

Here is how Varner described the makeup of the spermatozoa: "The head contains the nucleus, overlying acrosome (a membrane-bound compartment at the tip of the sperm head that contains enzymes to digest the outer surface of the egg), and a reduced complement of cytosolic (fluid portion of cytoplasm) elements. The head can be subdivided into an acrosomal region, equatorial segment, post-acrosomal region, and posterior ring, which demarcates the junctions between the head and flagellum. The posterior ring is the site of plasma membrane anchoring to the nuclear envelope and is thought to produce a tight seal that separates cytosolic components of the head and flagellum. The flagellum can be subdivided into a connecting piece, middle piece, principal piece, and end piece. These various parts of the spermatozoon are surrounded by a common plasma membrane."

Many of the sperm, Varner said, do not complete the journey and are lost along the way, with only about 0.0006% of spermatozoa gaining access to the oviducts following insemination.

The spermatozoa are virtually immobile in the epididymis, Varner told the group, but develop motility when ejaculated. After ejaculation, the sperm make their way from the uterus into the oviduct on a journey that allows them to meet up with the descending egg. Many of the sperm, Varner said, do not complete the journey and are lost along the way, with only about 0.0006% of spermatozoa gaining access to the oviducts following insemination. The remainder are lost through the cervix. Approximately four hours are required for sufficient sperm to ascend from the uterus into the oviduct to establish pregnancy at a normal rate.

The ejaculated spermatozoa cannot fertilize an egg right away. Varner described it this way: "In mammals, freshly ejaculated spermatozoa are not immediately capable of fertilizing an oocyte. Early studies showed that spermatozoa require residence time in the female reproductive tract to gain this capability, later termed capacitation."

The principal location where capacitation occurs, Varner said, is the caudal (toward the rear) segment of the oviduct. "Interactions with an ovulated oocyte, however, require spermatozoal migration to the ampullar region of the oviduct, and only a small percentage of spermatozoa that gain access into the oviduct will eventually arrive at this fertilization site. The precise mechanisms by which spermatozoa migrate to the ampullar region of the oviduct remain speculative, but contractile movements of the oviduct and hyperactivated spermatozoal motility are thought to play key roles in this migratory phase."

After capacitation, the spermatozoa must undergo an "acrosomal reaction" before they can pentrate the vestments of the egg required for fertilization. This is a rather complicated reaction that occurs upon spermatozoal contact with the zona pellucida, a thick, transparent outer envelope that encases an egg. In this reaction, the outer membrane of the acrosome fuses at multiple points with the overlying plasma membrane of the sperm head. The process enables the release of enzymes from the acrosome that are necessary to enable the sperm to enter the egg.

Once that entry has been made, the sperm's journey has come to an end. And so it was for "Dr. Dick," who was able to head off into the sunset, looking for new adventures.

Stallion Fertility Varner directed the final two hours to a discussion of stallion fertility and told the group, for example, that morphologically abnormal sperm often do not have a negative impact on normal sperm. "Therefore," he said, "the total number of morphologically normal sperm in ejaculates may provide more information regarding the fertility of a stallion than the percentage or absolute number of morphologically abnormal sperm."

He also discussed ways to refine a stallion breeding soundness examination, and he described newly developed laboratory assays that might improve the predictive value of such an examination.

Varner concluded his talk by telling the group that, while scientists have achieved much concerning research on sperm, more study is needed.

"Goals might include devising methods for long-term cooled semen preservation, improving the preservation of cryopreserved (frozen) semen, and incorporation of *in vitro* fertilization (both conventional *in vitro* fertilization—using multiple sperm—and intracytoplasmic sperm injection, where only one sperm is injected in the egg) in commercial programs," he said.

"Although these might seem to be lofty goals, a more absolute understanding of the spermatozoal structure and function would certainly take a lot of the 'guesswork' out of current approaches to analysis and manipulation of equine spermatozoa."

Perinatology

Traditionally, one of the stronger parts of each AAEP convention program is the time devoted to reproduction. The convention held in Orlando was no exception. It began with an in-depth session titled "Perinatology—End of Pregnancy Through Beginning of Life," during which experts in the field presented hour-long lectures on various reproductive problems, and it ended with two separate sessions of 20-minute lectures in which the presenters dealt with specific reproduction topics.

In-depth presenters were Wendy Vaala, VMD, Dipl. ACVIM, who is employed by Intervet Inc.; Margo Macpherson, DVM, MS, Dipl. ACT, of the University of Florida; Regina Turner, VMD, PhD, Dipl. ACT, of the University of Pennsylvania's New Bolton Center, and Robert Franklin, DVM, Dipl. ACVIM, a referral hospital veterinarian based in Ocala, Fla.

Late-Term Mare, Newborn Foal Leading off in the in-depth section and continuing on as moderator was Vaala. She offered "New Perspectives on the Late-Term Mare and Newborn Foal."

Under ideal conditions, Vaala told the group, the late-term mare would be managed before birth by a specialist in reproduction and after birth by a specialist in neonatology. However, she said, in most ambulatory practices, one person must be prepared to fill both roles, have proper equipment to assist in birth, and, if necessary, provide resuscitation and nursing care for the foal.

Many problems begin within the uterus, she told the group, and the practitioner must be able to monitor fetal development in order to determine early if something is going awry.

When a mare, especially one in the at-risk category, nears parturition it is important to monitor her progress so that help is at hand if needed. There are many monitoring aids available, she said, but no one aid should be considered infallible. "Nothing beats a human walking by and checking on the mare," said Vaala.

ANNE M. EBERHARDT

Observation and regular check-ups of the mare and foal are recommended post-parturition.

One of the problems that occur is prolonged gestation. It is important that the practitioner has a complete history of the mare, including whether she has been kept on fescue. There are some 35 million acres of fescue in the United States, Vaala said, and fescue toxicosis from infected varieties can lead to prolonged gestation and myriad other problems that can compromise the foal's chances for survival.

The drug of choice in dealing with fescue toxicosis, she said, is domperidone.

Once the foal has been delivered and the fetal membranes passed (something that should occur within three hours of delivery), Vaala told the group, it is very important that you save and weigh the placenta. The weight of a placenta, which can be 10-11% of fetal weight, can be an indicator that problems might exist with the newborn. Heavy placentas, she said, might be associated with conditions such as edema (fluid swelling), congestion, and/or infections. A light placenta might relate to incomplete development of the foal, along with other conditions.

Inducing labor in a late-term mare, Vaala said, should only be used as a last resort. "Indications for induction should only be limited to conditions that would seriously threaten maternal or fetal health if the pregnancy were allowed to continue, or if unsupervised, spontaneous delivery would occur," she said. "Examples of such conditions include hydrops (excessive placental fluids), prepubic tendon rupture (tearing of the ligament that supports the abdomen in the mare—loss of this structure makes it impossible for mares to contract muscles and deliver the foal normally), imminent death of the mare because of colic or other systemic illness, and maternal history of dystocia requiring mandatory assistance during foaling."

The quality of the mare's colostrum, which Vaala called "liquid gold," should be evaluated immediately after delivery, she said.

Vaala noted in her written paper: "Equine perinatology has evolved rapidly over the last two decades. However, most mares will continue to foal in locations other than in large clinics and university hospitals. Therefore, it will remain in the hands of clinicians in private practice to help improve our knowledge of the high-risk mare by using and reporting on the use of the antepartum (pre-foaling) monitoring techniques and interventional strategy discussed in this presentation."

High-risk mare Macpherson concentrated on "Identification and Management of the High-Risk Pregnant Mare" in her lecture. There are a number of conditions that can jeopardize the pregnant mare, she said, and the practitioner's challenge is to identify those problems, then find proper approaches to resolve them.

Three common conditions, she said, are

early udder development, acute pain, or an unusual increase in abdominal size. For each condition, she presented the case history of a mare that had suffered from the condition.

She first discussed premature mammary gland development. Normally, mares undergo udder development between two and four weeks before giving birth. Development of the udder prior to that might be an indication she is going to foal early, Macpherson said.

Two of the conditions that can cause early udder development are the presence of twins and bacterial placentitis (inflammation of the placenta), Macpherson told her listeners.

In the case of twins in late gestation, she said, the best approach has already been missed. That would involve early detection at 13 to 15 days gestation and the elimination of one embryo. Carrying twins to term puts the mare at risk for a number of complications, including dystocia (difficult birth). Once twins reach the late-term stage, she said, there are basically two options left for the practitioner: terminate the pregnancies, or allow the pregnancies to continue, monitor the mare, and provide assistance during delivery. Neither is particularly palatable to the practitioner, she said.

A promising approach for treatment of bacterial placentitis, Macpherson said, involves administering trimethoprim sulfamethoxazol and pentoxifylline in conjunction with altrenogest (Regu-Mate).

> **"Nothing beats a human walking by and checking on the mare."**
>
> DR. WENDY VAALA

In a study at the University of Florida, she said, 10 of 12 mares with experimentally induced placentitis delivered live foals after treatment with this protocol.

The colicky late-pregnancy mare poses a serious challenge for the practitioner, Macpherson said, because it first has to be determined whether the manifestation of pain is from labor contractions or some other condition not connected with the birthing process.

Once the diagnosis had been made, a treatment protocol can begin. It is important to have a complete health history of the mare, Macpherson said. For example, if she has a history of colic when not pregnant, there could be greater likelihood of her having gastrointestinal disease than a type of colic related to pregnancy.

The case study mare Macpherson described underwent colic surgery and four days later delivered a live foal.

A mare in late pregnancy that develops unusual abdominal distention is frequently in great jeopardy, Macpherson said, and she is at risk of losing the pregnancy. There are several conditions that could cause the problem, including hydrops, rupture of the prepubic tendon, and abdominal wall herniation.

Treatment options, she said, could involve use of a supportive belly bandage, induction of labor, and delivery by Caesarean section.

Three Problems Turner told the group that when she began preparing her presentation, she had decided to discuss her top 10 list of postpartum problems in mares. Then she realized that there wouldn't be enough for that lengthy of a presentation and cut it back to five, then to three.

The three problems she chose were 1) hemorrhage from uterine or ovarian vessels, 2) uterine lacerations, and 3) retained fetal membranes.

Hemorrhage from uterine or ovarian vessels usually occurs during the birthing process, Turner said. Older mares that have had a number of foals appear to be at greater risk.

Treatment of the condition, she said, can be difficult and confusing. The practitioner must decide whether to place the mare in a tranquil setting to lower stress

and blood pressure, or whether he or she should institute aggressive treatment that might increase stress and blood pressure.

"In most instances, as a minimum," she said, "we will place an indwelling venous catheter and begin the mare on volume replacement fluid therapy." She discussed some of the drugs that have been used to treat the condition and others that are not recommended.

The prognosis for recovery is variable, depending on the severity of the hemorrhage, she said. "Mild hemorrhage into the broad ligament (a band of tissue that helps suspend the uterus within the abdominal cavity) typically is associated with a good prognosis for recovery," she said. "However, severe hemorrhage into the abdomen or uterus can be acutely fatal."

If the mare survives, she said, a future pregnancy might result in a recurrence. If the mare is a valuable producer, Turner said, embryo transfer should be considered.

She then turned her attention to retained fetal membranes. In the mare, she said, it is considered that fetal membranes have been retained if they have not been passed in their entirety in three hours.

The condition can occur in any breed but it is most common with draft mares. Friesian mares, she said, are at a significantly higher risk, even after an uncomplicated delivery. She described one study where 54% of Friesian mares retained their fetal membranes after normal delivery.

When membranes are retained, Turner said, the treatment of choice by many practitioners involves administering oxytocin. Forceful removal is not a wise approach, but gentle pressure can be helpful, along with uterine lavage.

Proper management of a mare that undergoes Caesarean section is important for the mare's survival and future reproductive success, she said.

One of the problems involved with Caesarean section, Turner told the group, is that it often is resorted to only after prolonged attempts for a vaginal delivery. Delivery of a live foal after Caesarean section, she said, results in foal survival rates of 11-42% and survival of the foal after leaving the hospital is lower still at 5-31%.

"The wide range in foal survival consistently depends on duration of second-stage labor," she said. "Thus, whether or not a mare has a Caesarean section is not what determines the outcome for the foal. Rather, it is how long it takes for a foal to be delivered, regardless of delivery method—this is the critical factor."

She had this succinct advice for the practitioners in the room: "Refer early."

Mares that undergo Caesarean section and have good postoperative management often have pregnancy rates between 58-68% if bred back that year. If bred back the next year, the rate rises to about 72%.

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>
> DR. ROBERT FRANKLIN

High-risk foal Franklin closed out the in-depth session with a discussion on "Identification and treatment of the High-Risk Foal."

Early identification of high-risk foals is imperative so that successful treatment can be initiated in a timely manner, he told the group. However, identification is only the beginning of the process. The ambulatory veterinarian should be equipped with appropriate equipment for resuscitation, assisting with breathing, and increasing heart rate.

A valuable tool, Franklin said, is an instrument that measures lactate levels (in the blood). High lactate level can be an indicator that the foal is in the at-risk category.

One of the new approaches described at this AAEP convention involved equipping attendees at some lectures with keypads for responding to specific multiple-choice questions, which then were immediately tallied by computer. The keypad response in one instance provided evidence that very few veterinarians attending the session utilize the lactate-measuring machine. Franklin made a strong pitch for its use and cited a number of examples where the device had first identified at-risk foals, then was helpful in monitoring their recoveries.

A later presentation would underline his point that measuring lactate levels can be helpful at both the diagnostic and monitoring levels (see page 21).

Franklin said in conclusion: "It is obvious that early recognition of the high-risk or abnormal foal is the key to a successful outcome. Efforts and financial contributions should be made early in the course of the foal's life to document all problems so that an appropriate prognosis and treatment plan can be made. Mares with peripartum disease should be monitored closely for foaling trouble or for the delivery of a compromised foal. Preparations in terms of equipment for resuscitation, veterinary planning, and foaling attendance are critical to the successful delivery of a high-risk foal."

Reproduction

Fetal pulse Stefania Bucca, DVM, of XY Equine Veterinary Services in Kildare, Ireland, reported on using the fetal pulse rate as an aid in determining fetal health. Abnormal patterns in fetal heart rate, she said, are a clear indication of fetal compromise. The veterinarian can record the pulse rate with the use of transrectal ultrasound, she said.

Periparturient hemorrhage Carolyn Arnold, DVM, Dipl. ACVS, of Texas A&M, reported on a study of mares treated at Hagyard Equine Medical Institute near Lexington, Ky., for periparturient (occurring either shortly before, during, or shortly after parturition) hemorrhage. She said that 73 mares met criteria for inclusion in the study and that most of them had delivered a number of foals. Most of the mares, she told the group, were admitted to the hospital within 48 hours of giving birth and manifested signs of abdominal pain and hypovolemic shock (an affected horse has abnormally low levels of blood plasma in the body, such that the body can't properly maintain blood pressure, cardiac output, or normal amounts of fluid in tissues). The aim of treatment was to relieve pain, enhance coagulation (clotting), and restore perfusion (blood flow), she said. The survival rate of the treated mares was 84%. Mares that didn't survive in a number of instances were those that hemorrhaged before giving birth.

Hemorrhage in the field Charles F. Scoggin,

DVM, MS, of Pioneer Equine Hospital in Oakdale, Calif., continued the discussion on periparturient hemorrhage. He said in one study it was found that rupture of a uterine artery frequently was the cause of death in nonsurviving mares. Scoggin spent most of his lecture on various approaches the attending veterinarian might take when faced with a case of hemorrhage in the field. He emphasized that periparturient hemorrhage is a severe and life-threatening condition in mares and that immediate treatment is imperative. It is important, he said, to keep the mare as calm as possible.

Foal adoption Peter Daels, DVM, PhD, Dipl. ACT, ECAR, of Belgium, discussed approaches veterinarians can take to induce lactation in a mare and how to convince her to adopt an orphan foal. Work at the Keros Equine Insemination and Embryo Transfer Center in Passendale, Belgium, where he is headquartered, has been going on for five years. Lactation in a nonparturient mare, Daels said, can be induced during a two-week treatment period by administering progesterone, estrogen, and dopamine D2 antagonist (in this case, sulpride) on a daily basis, along with milking the mare regularly. Two drugs that helped to arouse maternal instincts that would allow for foal adoption were dinoprost and oxytocin. Under the approach used by Daels and his colleagues, adoption was expected to be completed in five days.

Lactate levels and prognosis Imogen S.F. Henderson, BVSc, of the Royal (Dick) School of Veterinary Medicine in Scotland, reported on the monitoring of lactate levels in newborn foals to help form a diagnosis, monitor response to treatment, and form an accurate prognosis. She and her colleagues reviewed the records of foals under 96 hours of age. It was found, she said, that nonsurvivors had significantly increased lactate levels at 12 to 36 hours of age as compared to survivors. A cutoff point of 4.85 mmol/L of lactate at admission correctly classified more than 80% of cases as either survivors or nonsurvivors, with the nonsurvivors being foals with lactate levels above the cutoff point. When lactate levels reached 11.3 mmol/L, she said, there were no survivors.

Glucose and lactate monitoring Pamela A. Wilkins, DVM, PhD, Dipl. ACVIM, ACVECC, of the University of Pennsylvania's New Bolton Center, concurred with Henderson's findings on using lactate levels in diagnosis and monitoring, but she added the measuring of glucose levels as another valuable tool. However, she also had a word of caution: "In both referral and practice situations, point-of-care (stall-side) glucose and lactate monitoring is inexpensive, easily performed, and potentially very clinically useful. However, individual monitors should be regularly compared with a standard laboratory technique because of variation in agreement when using horse blood or plasma. Understanding the potential limitations of any individual monitor used will aid in interpretation of the results produced by the meter."

Adrenal function Kelsey A. Hart, DVM, of the University of Georgia, told the group that evaluation of adrenal function is of growing importance in equine medicine. Hart discussed a study where the drug cosyntropin, synthetic adrenocorticotropic hormone (ACTH; natural ACTH is produced and secreted by the pituitary gland) was administered intravenously (IV) when low levels of cortisol were identified. "The results in this study," Hart said, "show that in 3- to 4-day-old foals, the cortisol response to IV administration of cosyntropin is dose-dependent, with higher cosyntropin doses resulting in a higher peak and longer duration of rise in cortisol."

Mathematical model Barton W. Rohrbach, VMD, MPH, Dipl. ACVPM (preventative medicine, epidemiology), of the University of Tennessee, presented a mathematical approach for predicting foal survival. He described the approach thusly: "By combining clinicians' experience based on the initial assessment of the foal with results of observations recorded for

a large number of hospitalized foals, the practitioner can provide a more precise estimate of the probability of foal survival."

Ovariohysterectomy

The final speaker in the Reproduction-Perinatology session was David E. Freeman, MVB, PhD, of the University of Illinois. He discussed performing an ovariohysterectomy (OHE) on mares. The surgery can be complete—involving removal of the entire uterus and ovaries—or incomplete with partial removal. The surgery, Freeman said, should be considered when a mare is suffering from a condition that is life-threatening, such as uterine torsion, or if she has a problem with a poor prognosis for resolution. Success rates are high and he pointed to one study that revealed 16 of 17 mares undergoing the procedure had survived. He said OHE is a viable option in certain instances as an alternative to ineffective medical treatment or euthanasia.

More Reproduction

One of the final sessions held at the AAEP convention involved further discussion on reproduction.

Advanced approach Silvia Colleoni, PhD, of the Laboratorio di Techologie della Reproduzione in Cremona, Italy, was the first speaker and discussed advances in ovum pick-up (OPU), intracytoplasmic sperm injection (ICSI), and embryo culture in equine practice. The process involves collecting immature oocytes from the ovaries, maturing them *in vitro*, injecting them with a single sperm (ICSI), and implanting the embryo—either in a fresh state or after having been frozen and thawed—into a recipient mare. The success rate has not been extremely high, but there has been success, she said.

She reported on a study that involved data collected from 47 commercial OPU sessions performed from 2004 to 2007 on 30 donor mares ranging in age from 3 to 24 years old. Here are the results: 808 follicles were aspirated and 474 oocytes recovered—a mean recovery rate of 58.19%. From this group, 66.05% reached a stage at which they were fertilized via ICSI, which resulted in 199 cleaved embryos and 40 blastocysts (the stage at which an embryo is ready for transfer). At the time of her report, 18 embryos had been surgically implanted and 15 of the recipient mares had become pregnant.

Making use of ICS Elaine M. Carnevale, DVM, MS, PhD, of Colorado State University (CSU), which pioneered the ICSI concept, reported on the success rate of the procedure during the 2006 breeding season at CSU. From 91 normal oocytes, she said, 62 cleaved into at least two cells and were fertilized with one of the following: fresh, cooled, or frozen semen. After ICSI, the embryos were transferred, achieving a pregnancy rate of 44% at 16 days and 31% at 50 days. The type of sperm injected had no effect on the outcome, she said. Using ICSI, Carnevale told the group, 24 late-term pregnancies were produced for stallions with poor-quality semen or limited sperm numbers.

Intervals between mating and ovulation of more than two days significantly lowered pregnancy rates in Thoroughbred mares.

DR. TERRY BLANCHARD

FSH hormone Kory D. Niswender, DVM, MS, Dipl. ACT, of Reata Equine Hospital in Weatherford, Texas, reported on the use of recombinant equine follicle stimulating hormone (reFSH) to promote follicular development in cycling mares. His conclusion was that reFSH "may be a useful tool to stimulate follicular development in the mare."

Using hCG Jodyne Green, DVM, of the Western College of Veterinary Medicine at the University of Saskatchewan, reported on the use of human chorionic gonadotrophin (hCG) as a stimulant for ovulation. Green said results of a retrospective study showed: "In summary, hCG is effective in inducing ovulation in 73% of mares administered this drug. Follicular size and cervical tone at time of treatment with hCG are associated with ovulation within the next 48 hours. A decrease in endometrial edema (excess fluid swelling) score from the initial administration of hCG to 24 hours was associated with ovulation in the 24- to 48-hour window. As the breeding season progresses, there is a reduction in the ovulatory response rate to hCG administration related to increasing number of treatments and seasonal factors. In the fall, fewer mares ovulate in the 24- to 48-hour window. Practitioners may consider altering monitoring schedules based on the season, number of hCG treatments, and changes in endometrial edema."

Breeding and ovulation interval Terry Blanchard, DVM, MS, Dipl. ACT, formerly of the Texas A&M University faculty and now a veterinarian at Hill 'n' Dale Farm in Kentucky, told the group that intervals between mating and ovulation of more than two days significantly lowered pregnancy rates in Thoroughbred mares. It would be wise, he said, to breed the mare again "double service" (mate again on the same estrus) if she has not ovulated within two days of being bred the first time.

Interpreting endometrial edema Juan Samper, DVM, MSc, PhD, Dipl. ACT, of Veterinary Reproductive Services in Langley, British Columbia, told the group that when a mare is under the influence of estrogen, there is increased blood flow to the uterus that results in an increase in endometrial edema. The edema can be viewed ultrasonographically, he said, describing a measuring system with values from 0 to 5 to assess the edema. The bottom end of the scale would normally exist in mares that were in a state of diestrus (not in heat), and the higher end is when mares are in estrus. During examination there are several instances in which the veterinarian should be alerted to possible problems, Samper said. He identified them as: 1) presence of obvious endometrial edema and a large follicle 14-15 days after ovulation; 2) presence of hyper-edema during the normal estrous period; 3) failure to reduce edema as the mare approaches ovulation and the presence of marked uterine edema 24 hours after ovulation; 4) significant increase in the degree of uterine edema 12 to 24 hours after breeding; and 5) lack of uterine edema during the estrous period. "Interpretation of endometrial edema," he said, "requires a good-quality ultrasound and evaluation of the mare on a regular basis during the late diestrous and early estrous period until ovulation is detected."

Measuring progesterone Edward L. Squires, MS, PhD, of CSU discussed the best approach to take when measuring progesterone (hormone that supports a

new pregnancy) of the mare during early gestation after nonsurgical embryo transfer. An appropriate progesterone level, he pointed out, is highly important in maintaining pregnancy. Squires reported on a study that involved measuring progesterone levels in blood that was collected once a day, morning and night on a given day, and once a day for two days. Here is the conclusion: "Because the variance was quite small between the morning and evening samples on a given day, there did not seem to be any major advantage to collecting samples twice in a given day. In contrast, the variance was greater for samples collected two days in a row. This indicates that two daily samples provide a better estimate of the mare values than one sample. In other words, if the clinician bleeds (collects blood from) the mare two days in a row, the accuracy of the estimate can be increased considerably over obtaining just one individual sample.

Anestrus Claire Card, DVM, PhD, of the University of Saskatchewan, reported on a study to determine whether administering a vaccine against gonadotrophin-releasing factor (GnRF) would prevent mares from coming into estrus. Mares in the study received two injections of GnRF vaccine four weeks apart. In the study, it was found that four weeks after the second dose, 98% of the mares receiving GnRF were anestrous or transitional. The treated mares ceased cycling for the rest of the breeding season. The next year, 88% percent of the treated mares were cycling, and the year after that, 98% were cycling. Pregnancy rates for the treated mares in the first year after vaccination were 75% and rose to 90% the following year.

Oxytocin to block luteolysis Dirk Vanderwall, DVM, PhD, Dipl. ACT, of the University of Idaho, reported on the use of oxytocin to successfully block luteolysis (destruction of the corpus luteum), thus, prolonging the lifespan of the corpus luteum. The importance of a functioning corpus luteum, he reminded the group, is the production of progesterone. Administering 60 units of oxytocin twice daily on Days 7 to 14 post-ovulation blocked luteolysis and induced prolonged corpus luteum function, he said. Mares with prolonged luteal function, he told the group, maintained progesterone levels of more than 1.0 ng/ml continuously through Day 30. This level of progesterone, he said, is sufficient to block estrous behavior, "therefore, disrupting luteolysis by administering exogenous oxytocin seems to be a plausible method of long-term suppression of estrus in mares."

Paraphimosis in the stallion Steven Brinsko, DVM, MS, PhD, Dipl. ACT, of Texas A&M University, discussed the condition known a paraphimosis, where the stallion is unable to retract his penis. The condition most often surfaces in breeding stallions, Brinsko said, but it can also occur in geldings. It begins with penile prolapse (protrusion or displacement), and that results in excessive edema and swelling of the penis and prepuce. Early, aggressive therapy is important, he said, because it can minimize secondary complications. The primary goal, he told the group, is to reduce swelling and replace the prolapsed penis into the preputial cavity as soon as possible. The use of anti-inflammatory drugs and hydrotherapy are part of the treatment protocol, as is penile support to prevent further swelling.

Post-mortem sperm collection Adam C. Eichelberger, DVM, of the University of Florida, concluded the session by discussing how to collect, handle, and process semen from the epididymis of a stallion that has died or has been euthanatized. Being able to harvest sperm from a dead stallion can be important, he said, because reproductive science has produced ways in which to preserve the sperm for later use. He discussed the approaches a veterinarian should take in first obtaining the testicles, then harvesting the sperm residing there. He said he and his colleagues have "collected a range of sperm from 5 to 18 billion per testicle."