

## POST-THAWS

The foal below, born in 2001, was conceived by frozen semen from the Standardbred stallion Western Hanover via embryo transfer performed by Dr. John G. Shaw, Prebbleton Farm, New Zealand.



Post-thaws is reserved for photos of foals resulting from breedings from semen frozen by Select Breeders Services laboratories. If you have a photo you would like included in our newsletter please contact our main office at (877) 658-3328 or email us at [info@selectbreeders.com](mailto:info@selectbreeders.com).

## I N S I D E

Technology to the rescue:  
The Kid Clue story

Low dose insemination

New scheme for  
managing mares for  
frozen semen breedings

SBS Affiliated Laboratory Network  
NEW laboratories added in 2001



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Winter 2001-2002

# FOUNDATIONS

## TECHNOLOGY TO THE RESCUE

### The Kid Clue Story

by Phil Matthews, DVM  
Peterson & Smith Equine Reproduction Center



Quarter Horse Stallion  
KID CLUE

Obviously, most breeders think their stallion's semen is "precious", but what if their stallion has proven himself to be the best sire within his discipline, as well as a sire of sires and now he's dead! Now precious becomes an apt description.

KID CLUE, the most prolific and popular Quarter Horse halter stallion, died in January 2001. The Perry family had decided to freeze the semen of this horse in the fall of 2000. Most of their motivation was to create some "slack" for him during the busy breeding season. This would be accomplished by having frozen semen available on heavy breeding days when there may not be enough semen to be handled by a single ejaculate; or conversely on very light days so that the horse may not need to be collected at all.

Luckily, this horse's semen froze very well. This was somewhat surprising in that his fresh semen wasn't stellar, and didn't ship well either. He makes a great case for test freezing any stallion. In fact this is the only way to ship his semen successfully.

DUE TO THE CIRCUMSTANCES that made his semen even more precious, we wanted to ensure that none was wasted. We elected to do low dose endoscopic insemination using only two (0.5 ml) straws of semen at each insemination. (With this technique even two straws is probably "overkill"). Two straws constitutes one quarter of the normal insemination dose.

AT PETERSON & SMITH EQUINE Practice we have progressed from the antiquated theory that a mare needs to be palpated 3 or 4 times daily when breeding with frozen semen. Our general protocol, which was put to use here, is to palpate/ultrasound mares once daily and administer hCG or Ovuplant once a follicle has reached 32 to 36 mm. The mares are inseminated at 24 and 42 hours after administration of the ovulatory agent. If a mare hasn't ovulated by the second insemination, she is inseminated again 24 hours later. (Approximately 9% of the mares were bred additional times due to the fact that they didn't

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# RESEARCH & DEVELOPMENT RESEARCH & DEVELOPMENT

## NEW SIMPLE AND EFFECTIVE MANAGEMENT SCHEME FOR INSEMINATION OF MARES WITH FROZEN SEMEN

One of the most common objections to the use of frozen semen is the increased cost to the mare owner associated with frequent ultrasound exams, often including "midnight exams" in an attempt to inseminate mares as close to ovulation as possible. It is generally accepted that optimum conception rates with frozen semen are obtained when viable sperm are inseminated in the period of 12 hours prior to 6 hours after ovulation. When frozen semen is marketed without guarantee of pregnancy and sold "by the dose" or as only a few doses per breeding fee, veterinarians will try to conserve doses by insemination of only a single dose of semen within this period. Since predicting ovulation within such a short time frame is very difficult for even the most experienced clinicians, frequent examinations of the growing follicle (often every 6 hours) is performed so that a single insemination can be made as soon as ovulation is detected. This minimizes the number of doses used and ensures that insemination occurs within the recommended time period relative to ovulation. Unfortunately, this scheme requires examinations every 6 hours, which are expensive for the mare owner and time consuming and impractical for the veterinarian.

SELECT BREEDERS SERVICE, INC. encourages stallion owners to adopt breeding contracts that provide ample doses of frozen semen to employ a new simpler insemination scheme. This new protocol is much more cost effective for mare owners and allows veterinarians to manage mares for insemination with frozen semen in a manner similar to that used for cooled semen. The protocol involves

single daily examinations, use of the ovulation-inducing agents hCG or Ovuplant, and 2 inseminations timed to occur at specific time intervals from administration of the ovulatory agent. This protocol has been tested in both clinical and laboratory fertility trials and has proven to be as effective as more expensive and labor-intensive protocols. All shipments of frozen semen distributed through Select Breeders Service will include the management recommendations that appear in the Breeding Managers Forum on page 3 of this edition of FOUNDATIONS.

### RESULTS OBTAINED USING THE NEW INSEMINATION SCHEME

SELECT BREEDERS SERVICE EUROPE:  
CLINICAL TRIAL 2000 & 2001

A COMPARISON WAS MADE between fertility obtained when two insemination schemes were used to inseminate mares with frozen semen. Frozen semen from each of 12 stallions was used to inseminate mares either once per cycle, in the period within 6 hours immediately after ovulation, or twice per cycle, 24 and 40 hours post-hCG treatment. The single AI scheme required ultrasound examinations every 6 hrs during the periovulatory period and resulted in first cycle and seasonal pregnancy rates of 70.6% and 82.8% (24/29), respectively. The two-insemination protocol required only once daily ultrasound examinations and resulted in first cycle and seasonal pregnancy rates of 78.3% and 92.3% (36/39), respectively. These pregnancy rates are very high and reflect the fact that the stallions were selected based on known good fertility with frozen semen.

*continued on page 4*

## Evaluation of Frozen-Thawed Stallion Sperm

Emily Kirk  
Select Breeders Service  
Colorado, Maryland

The acceptance of foals conceived from frozen-thawed sperm by most international horse breed associations has enhanced the interest and demand for frozen semen. Although frozen semen provides unique advantages to horse breeders, this technology does not come without disadvantages. Freezing semen increases the potential for fragile sperm cells to be damaged during processing, threatening the original viability and fertility of the semen sample. A sperm must possess multiple attributes to be fertile, and each of these attributes must be expressed at the correct time and place. Many of the sperm attributes necessary for fertility are known, but perhaps some are unknown. A partial list of these attributes include progressive motility, metabolism for energy production, acceptable morphology, plasma membrane integrity, the ability to capacitate, acrosome react and bind to an oocyte, and a functional complement of DNA. These sperm attributes can act in a synergistic, additive or antagonistic manner and it is probably their combined effectiveness that contributes to fertility.

Motility is the most common method used to evaluate the quality of equine sperm, although post-thaw motility is not always representative of the fertilizing potential of a sperm sample. Damage that occurs to sperm during the freeze-thaw process can be subtle and not adversely affect motility. As increasing numbers of horse breeders take advantage of the convenience of frozen semen, it becomes increasingly important to develop reliable assays to evaluate its potential fertility. These assays could save time, money, and frustration by identifying sub-fertile sperm samples prior to their use in breeding programs. Analysis of frozen-thawed sperm was the

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# THE BREEDING MANAGERS FORUM

## Step by Step Management of Mares with Frozen Semen

■ Verify that the mare is a suitable candidate for AI with frozen semen by performing a routine reproductive exam that may include culture, cytology and biopsy when indicated. Data suggests that aged (>15 years) or repeatedly barren mares will have a significantly reduced pregnancy rate and should not be selected for use with frozen semen.

■ Once the mare comes into estrus, palpate and/or scan daily to monitor follicular activity.

■ Upon detection of a large (35-40 mm) pre-ovulatory follicle, administer hCG or Ovuplant.

1. If more than one dose of semen is available for insemination on a given heat cycle:

- Continue to examine the mare via ultrasound once daily and inseminate a single dose of frozen semen approximately 24 hours after hCG administration.
- Examine the mare approximately 16 hours after insemination and inseminate a second dose of frozen semen even if the mare has already ovulated.
- Examine the mare the following day to confirm ovulation. Insemination of a third dose may be required if the mare has still not ovulated.

*Note:* A general goal for mares inseminated with frozen semen is to inseminate within 12 hrs prior to or within 6 hrs after ovulation. This protocol insures that viable sperm are in the oviduct during that interval for any mare that ovulates within a period of 18 to 52 hours following administration of hCG or Ovuplant.

2. If only one dose of semen is available for insemination:

- Examine the mare via ultrasound at 6-hour intervals starting 12-24 hours after hCG or Ovuplant administration.
- Inseminate the single dose of frozen semen as soon as ovulation is detected. It is extremely important that mares being inseminated post-ovulation are inseminated within 6 hours of ovulation. A significant reduction in fertility will occur if mares are inseminated more than 6 hours post ovulation.

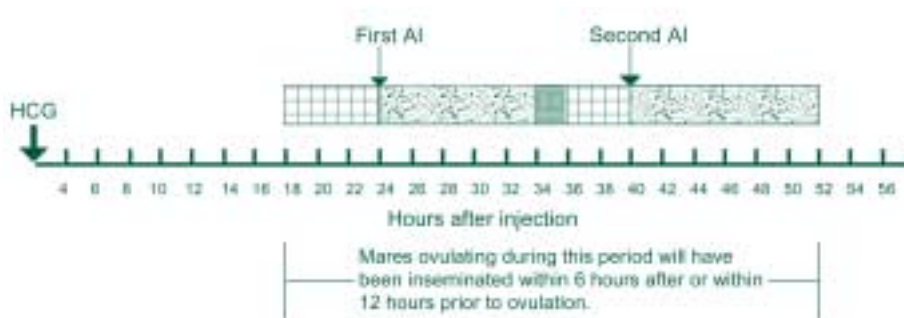
■ Mares that exhibit a delayed clearance of post-mating induced fluid should be treated with oxytocin (greater than 4 hours after each breeding or after the last breeding) and/or uterine lavage to aid in the mechanical expulsion of fluid from the uterus.

Suggested schedule for insemination of mares with frozen semen when more than one dose is available:

Daily examinations during estrus (any time)

Day 0: Day of 35 to 40 mm follicle detection - Administer hCG or Ovuplant (deslorelin) at approximately 4:00 PM

Day 1: Inseminate a single dose of frozen semen at 4:00 PM (24 hrs post injection)



Day 2: Inseminate a second single dose of frozen semen at 8:00 AM (40 hrs post injection)

Day 3: Examine to confirm ovulation and inseminate a third dose if the mare has not ovulated at 8:00 AM



## New Management Scheme

*continued from page 2*

COLORADO STATE UNIVERSITY EXPERIMENT 2001

ONE OF THE ARGUMENTS against breeding mares twice during a given cycle is the increased use of semen. Therefore, a study was conducted at Colorado State University to compare insemination of mares with 800 million total sperm within 6 hours post-ovulation, versus insemination of mares 24 and 40 hours after hCG administration using 400 million total sperm (half a normal dose). Forty normally cycling mares were synchronized with progesterone and prostaglandins.

The timed insemination certainly was a more practical breeding scheme since mares were only examined once daily after hCG administration.

Upon return to estrus, mares were randomly assigned to one of the two groups. Mares were examined once daily until a 35-mm follicle was detected. Those to be bred post-ovulation were given hCG and examined the following day. Subsequently, mares in this group were examined 4 times a day until the time of ovulation and then inseminated within 6 hours post-ovulation with 800 million sperm cells. Mares in the other group were given hCG once a 35-mm follicle was detected and then examined only once daily throughout estrus until the time of ovulation. They were inseminated with 400 million sperm cells at 24 and 40 hours after hCG. Semen from 3 stallions was frozen

in a lactose-EDTA extender at a concentration of  $400 \times 10^6$  sperm/ml in .5-ml straws. The semen from each stallion was used to breed 13 or 14 mares.

EIGHT DAYS AFTER OVULATION, embryo recovery was attempted from each mare as an indication of fertility. Pregnancy rates were similar in both groups. For mares inseminated once only post-ovulation, 12 of 23 mares provided an embryo (60%). Those in group 2, inseminated 24 and 40 hours post-hCG with 400 million spermatozoa, provided 11 embryos out of 20 attempts (55%).

THUS, BASED ON THESE DATA, we concluded that breeding mares at a fixed time after hCG (24 and 40 hours) with a half dose (400 million total spermatozoa) resulted in similar fertility to those mares bred with a full dose (800 million total spermatozoa) within 6 hours post-ovulation. The timed insemination certainly was a more practical breeding scheme since mares were only examined once daily after hCG administration. Thus, this scheme of timed insemination required fewer examinations of the mare and used the same number of sperm as those inseminated post-ovulation. ■

## Evaluation - *continued from page 2*

focus of the research for my masters degree at Colorado State University, where I developed and validated an assay to evaluate the sperm plasma membrane, acrosome, and mitochondrial function. This assay utilized fluorescent dyes that target specific sperm compartments. The sperm samples were evaluated using a flow cytometer, an instrument that rapidly and objectively detects the fluorescence of individual sperm.

Analysis of sperm using the flow cytometer was found to have less variability than motility analysis. Similar to the automated motility analyzers used by Select Breeders Service, the flow cytometer eliminates variability caused by human bias. In addition, the flow cytometer can evaluate thousands of sperm compared to only several hundred sperm evaluated during a motility analysis. The aim of this assay was to obtain some information about sperm quality in addition to sperm motility. It was found that some sperm samples, especially those possessing very low post-thaw progressive motility, had integrity of sperm compartments much higher than expected. These samples contained a large percentage of live but presently non-motile sperm, but it is not known how fertility is affected by this phenomenon because the fertility of these samples was not tested.

Further investigation was conducted using frozen semen with known fertility, provided by Select Breeders Service, Inc. From this study it was concluded that analysis of multiple sperm attributes, including a combination of sperm

motility measured at 90 minutes post-thaw and the integrity of sperm compartments measured by the flow cytometer, best explained the differences in fertility between stallions. Combining results from the analysis of multiple sperm attributes evaluates the fertilizing potential of frozen-thawed sperm better than if only a single parameter is considered. Unfortunately, our analysis did not identify a possible cause of subfertility of two stallions whose frozen semen obtained no pregnancies. Although sperm aberrations not measured in this study can cause infertility, the new flow cytometric assay provides an objective and reliable method to evaluate sperm attributes and provides additional information about the potential fertilizing capacity of sperm. ■

...it was concluded that analysis of multiple sperm attributes, including motility and integrity of sperm compartments best explained the differences in fertility between stallions.



**WITH 18 YEARS OF EXPERIENCE** in the commercial production and distribution of frozen semen, Paul Loomis, founder of Select Breeders Service, Inc. has a vested interest in the responsible development of the frozen semen industry. Growth of the equine frozen semen industry has been slow for a number of reasons. First, the lack of quality control by many facilities producing frozen semen has resulted in very variable results following insemination. Second, not all stallions produce semen that can retain acceptable fertility after freezing and thawing and therefore should not be used in a frozen semen program. Third, International distribution of frozen semen has traditionally been marketed on a "fee per dose" basis with no fertility guarantees. This places an unfair share of the risk of obtaining a pregnancy on the mare owner. Also, it limits the number of doses that the veterinarian has to work with to obtain a pregnancy and results in the practice of inseminating only one dose per heat cycle. When this is the case, veterinarians are trying to inseminate very close to ovulation requiring intensive mare management and

resulting in greatly increased costs to the mare owner. Providing mare owners with at least 2 doses per cycle and backing the product with a fertility guarantee (similar to any other form of breeding) will stimulate the use of frozen semen and improve results.

SBS has developed a network of laboratories worldwide that have experience, facilities and equipment for cryopreservation and distribution of equine semen. Personnel from all network laboratories have been trained in the techniques for semen cryopreservation that have been developed by SBS and employ controlled rate cell freezers for precise and consistent control of freezing protocols. Central laboratories in North America and Europe (also planned for Australia and South America) provide extenders and also perform post thaw quality control for semen frozen by numerous Affiliated Laboratories throughout the region. All post-thaw quality control evaluations of sperm motility are performed using computer assisted sperm analysis. The main reasons for the establish-

## The SBS Affiliate Laboratory Network

ment of this network are:

1. To establish and implement quality standards for frozen semen produced for commercial distribution within the breeding industry.
2. To provide breeders with an International system of distribution for frozen semen that promotes the concept of marketing semen with some form of fertility guarantees. An inventory of frozen semen can be shipped with confidence to an SBS Affiliate in another country, where it can be managed to maximize results and minimize risk to the stallion owner.
3. To consistently improve frozen semen technology through combined research and development and analysis of fertility data.

There are currently six laboratories in North America, two in Italy, one in Germany and one in Australia.

NEW NEW NEW NEW NEW NEW NEW NEW NEW NEW NEW

In 2001 Select Breeders Services welcomed FOUR NEW laboratories into its Affiliated Laboratory Network.

SBS Northgermany  
Muhlen, Germany

Pioneer Equine Hospital  
Oakdale, California

Gumz Farms  
North Judson, Indiana

SBS Australia  
in partnership with Gouldburn Valley Equine Hospital  
Shepparton, Victoria, Australia

Look for more information on these new laboratories in this and upcoming editions of FOUNDATIONS.

### SBS Northgermany

Tierärztliche Klinik für Pferde  
Munsterlandstrabe 51  
49439 Muhlen GERMANY  
49 5438 958560  
kontakt@sbs-northgermany.de

SBS NORTHGERMANY was established in the spring 2001. Located in north Germany, the mission of SBS Northgermany is to offer the highest quality equine semen



freezing and related services in Germany in collaboration with the SBS worldwide network.

TIERÄRZTLICHE KLINIK FÜR PFERDE was founded in the early 1990's. The clinic currently has four partners, Marc Koene, Jan-Hein Swagemakers, Kirsten Schwenzer and Julius Wegert. In 2001 the clinic moved into a new state-of-the-art facility. This new clinic is more than 400 square meters in size and is located in the heart of Germany's horse country, 6 miles east of Vechta.

As an SBSE Affiliate Laboratory SBS Northgermany offers equine semen freezing, storage and distribution services. For more infor-

mation contact Dr. Kirsten Schwenzer.

Along with the semen freezing services offered by SBS Northgermany the clinic offers breeding soundness examination of mares and stallions, reproductive management of mares with fresh, cooled and frozen semen, foaling assistance and intensive care, subfertility diagnosis and treatment of mares and stallions, embryo transfer and endoscopic insemination.

TIERÄRZTLICHE KLINIK FÜR PFERDE is also a full-service equine veterinary hospital offering other specialities including a variety of surgical services and diagnostic imaging services. ■

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## Gumz Farms, LLC

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NESTLED AMONG 200 ACRES in beautiful Northwestern Indiana, Gumz Farms is a complete AI facility. Working in cooperation with Dr. Brian Biggers of Biggers Equine Medical Services the experienced technicians at Gumz Farms receive cooled and frozen semen for on-farm breeding of mares. Stallion services include; training to the phantom, semen collection and evaluation, computerized analysis of sperm motility and processing for cooled shipment in our state-of-the-art laboratory. Semen freezing, storage

and distribution services are offered as part of the Select Breeders Services Network. Gumz Farms also boasts an impressive foaling facility designed with safety and comfort for the mare and foal in mind. Included is a neo-natal unit complete with cameras and 24-hour monitoring by experienced attendants for any unexpected problems or routine work.

Gumz Farms owned and operated by Amy and Kevin Gumz and is centrally located in North Judson, Indiana. Two hours northwest of Indianapolis and two hours southeast of Chicago.

## Pioneer Equine Hospital

11501 Pioneer Ave.  
Oakdale, California 95361  
(209)847-5951  
dvmba@pioneerequine.com

INCORPORATED IN 1978, Pioneer Equine Hospital, owned by Dr Jerry Black and Dr Brad Jackman, is a referral practice with five associate veterinarians (two ACVS Diplomates) and two interns. Referrals arrive from California, Oregon, Nevada, Utah and Arizona. Oakdale, CA is located in eastern San Joaquin valley, 80 miles south of Sacramento, 90 miles east of San Francisco and 60 miles west of

Yosemite National Park

Services include surgery, diagnostics and treatment of lameness, sport horse medicine and evaluation, resident farriers and reproductive services

Select Breeders Service Affiliate Lab Staff

Dr Jerry Black

Dr Hilda Baisel

Dr Duncan Peters

Reproductive services include Select Breeders Service, Inc. affiliated laboratory; pre-breeding evaluation of mares and stallions; cooled, transported semen services; mare foaling care and neonatal foal care.

## LATEST NEWS

### Select Breeders Southwest, Inc.

APPROVAL FOR THE USE of frozen semen by the American Quarter Horse Association and the American Paint Horse Association has kept the staff at Select Breeders Southwest, Inc. on their toes. Since the approvals we have done test freezes on over 100 American Quarter Horse and over 10 American Paint Horse stallions.

With the approval of frozen semen, the international market was opened up for these registries. In the past 12 months we have exported semen from 22 American Quarter Horses and 2 American Paint Horses. Destinations include Italy, Germany, Brazil

and Australia. We anticipate many more international shipments in 2002.

There have been several renovations to the Texas Facility in 2001. We have updated our breeding shed with a new mare tease rail and installed floor mats for stable footing in the breeding shed.

Erin and Whit have taken turns traveling with the mobile lab this year. In 2001 the mobile lab has been to North Carolina, South Carolina, Kentucky, Michigan, Ohio and Georgia. It has also been on many trips to South and West Texas. This fall the mobile lab will travel to Wisconsin as well as several places in Oklahoma and Texas. Although the interest in other states exists, a firm schedule has not been set. Please contact our office for the most up-to-date schedule.

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# Calendar of Events

## West Coast Equine Reproduction Symposium

November 29 - December 2, 2001  
Solvang, California

The West Coast Equine Reproduction Symposium is to be held in Solvang, California Nov. 29 through Dec. 2, 2001, immediately after the American Association of Equine Practitioners meeting. This meeting is open to all breeders and veterinarians. Details and registration information for the meeting can be found at the following website address:

<http://animalscience.ucdavis.edu/EquineSymposium>

Select Breeders is one of the many corporate sponsors for this meeting. Proceeds from this meeting are to be used for support of the International Symposium on Equine Reproduction.

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### Recent & Upcoming Events Sponsored by Select Breeders Services

3rd Annual International Symposium on Stallion Reproduction - 2001  
West Coast Equine Reproduction Seminar - 2001  
2nd Annual Texas Breeders Forum - 2001  
8th Annual International Symposium on Equine Reproduction - 2002

## Eighth International Symposium on Equine Reproduction

July 21-26, 2002  
Fort Collins, Colorado

The Eighth International Symposium on Equine Reproduction will be held at Colorado State University, Fort Collins, CO on July 21-26, 2002. This meeting is a forum for practitioners and scientists from around the world to present the latest information on equine reproduction.

Previous meetings have been held in England, California, Australia, Canada, France, Brazil and South Africa. Both oral and poster presentations will be given at this meeting. Research results will be presented in the following sessions: Stallion Reproduction, Non-pregnant Mare, Pregnant Mare, Perinatal and Gamete Biology. In addition, on 2 nights during the conference, workshops will be held on some of the most pressing areas of equine reproduction. Proceedings for this symposium will be published in *Theriogenology*.

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## Select Breeders Southwest Hosts 2nd Annual Texas Breeders Forum

Over 100 veterinarians, stallion and mare owners attended.

**the** 2nd Annual Texas Breeders Forum was held on August 3 and 4, 2001 at the Spirit of the West Guest Ranch and Executive retreat in Tioga, TX. Over 100 veterinarians, stallion and mare owners took the opportunity to listen to talks on equine behavior, semen evaluation, and frozen semen.

A special forum held for veterinarians only took place on August 3, 2001. This was an opportunity for veterinarians from Oklahoma and North Texas to hear abbreviated versions of the talks which would be given on Saturday. After the talks a panel discussion was used for the veterinarians to ask the

speakers about specific issues they may be encountering.

The day began with informative talks on equine frozen semen. Representatives from the American Quarter Horse Association, American Paint Horse Association and Arabian Horse Registry of America presented their perspectives on cooled and frozen equine semen and how it's affected their respective registries. The 2001 breeding season was the first time Quarter Horse and Paint Horse owners could utilize frozen semen.

Other speakers included Sandro Barbacini, DVM, Select Breeders Europe; Sue McDonnell, University of Pennsylvania; Dickson Varner,

DVM, Professor and Chief of Theriogenology at Texas A&M University; Ed Squires, Ph.D., Supervisor of the Equine Reproduction Laboratory at Colorado State University; Charlie Love, DVM, Texas A&M University; Allison Lindsey, MS, of XY, Inc.; and Whit Byers, MS, and Erin Bishop, MS, Select Breeders Southwest, Inc.

When the learning experience came to an end it was time for the evening's entertainment to begin. Dr. Squires, Dr. Varner, Allison Lindsey, Danny Salsman (Salsman Quarter Horses; Pilot Point, TX), Megan Leahy (Cedar Ridge Stallion Station; Whitesboro, TX) and Lisa

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# FROSTBITES

PAUL LOOMIS WAS AN INVITED SPEAKER at the 3rd International Symposium on Stallion Reproduction in Fort Collins, Colorado, January 10-12, 2001. The title of his presentation was: "Recent Advances in the Equine Frozen Semen Industry".

DR. SQUIRES VISITED INRA IN NOUZILLY, FRANCE and presented information on cooled and frozen semen at a conference on Utilization of Cooled and Frozen Semen. He also presented similar talks to the Society of Italian Equine Practitioners in Reggio Emilia, Italy. Dr. Barlucinne was the program organizer and host. The meeting was held concurrent to the Quarter Horse Reining Derby at Salone del Cavalho Americano

DR. SANDRO BARBACINI of Select Breeders Service Europe and Studio Veterinario Cristella presented a talk on the "Management of mares with frozen semen" at the 14th International Congress on Animal Reproduction in Stockholm, Sweden, July 2-6 2001.

DR. BARBACINI AND DR. NECCHI & ZAVAGLIA presented "Effect of post-insemination fluid accumulation on fertility of mares inseminated with frozen/thawed semen" at the 7th WEVA congress Sorrento, Italy - October 5-7, 2001.

## AAEP 2002

Paul Loomis is an invited speaker at the 47th Annual Convention of the American Association of Equine Practitioners in San Diego, California, November 24-28, 2001. The title of his presentation: "Storage, Handling and Distribution of Frozen Equine Semen".

Dr Phil Matthews of Peterson & Smith Equine Reproduction Center in Ocala, FL (an SBS Affiliate Laboratory) and Dr Dean Neely of Mid-Atlantic Equine in Ringoes, NJ will be moderating a "Table Topics" discussion at the AAEP on "Breeding Management with Frozen Semen".

BASED ON THE ARTICLE IN THE LAST SBS NEWSLETTER, there was some confusion as to Dr. Squires's role at Colorado State University. He is still a faculty member at Colorado State University and will continue in that capacity. He serves as a consultant for Select Breeders Service.

DR ED SQUIRES AND PAUL LOOMIS TRAVELED TO Shepparton, Victoria, Australia in August of 2001 at the invitation of Dr's Angus McKinnon and Jim Vassey of the Goulburn Valley Equine Hospital (an SBS Affiliate Laboratory) to speak on a variety of topics related to equine reproduction at the 2001 Breeders and Veterinarians Shortcourses.

PAUL LOOMIS HAS BEEN INVITED to speak at the 2002 National Association of Animal Breeders (NAAB) Technical Conference to be held in Milwaukee, WI on August 23-24, 2002. He has been asked to give a presentation entitled "Update on Equine AI".

DR NANCY COOK OF ADVANCED EQUINE REPRODUCTION in Solvang, CA (an SBS Affiliate Laboratory) will be an invited speaker at the West Coast Equine Reproduction Seminar in Solvang on November 29, 2001. Her topic will be "Managing Frozen Semen: Storage, Handling and Distribution".

## Special Announcement

On September 15, 2001, Erin Crockett was married to Phil Bishop in a lovely evening ceremony in Topeka, Kansas. The rain storms that permeated the day abated in time for the church ceremony and the fun reception that followed. A honeymoon in Puerto Vallarta was an ideal spot for R&R (Rest & Relaxation & Romance) - at least that's what the bride and groom report and the photos indicate.

## Texas Breeders Forum - *continued from page 7*

Moden (Kyle Ranch; Whitesboro, TX) participated in a highly competitive Lemon Race. The object of the race was to move their lemon on the floor over the finish line. The tool with which they moved the lemon was another lemon that had been put in the foot of a pair of pantyhose which had been tied around their waist. The pantyhose leg with lemon hung down in the front of the participant while the other leg and was tied at the waist using the remainder of the pantyhose. Got that picture? The gyrations necessary to create the "swing" were very creative. Everyone was a good sport and although no lemons were injured during the event a few egos may have been temporarily frayed. After the lemon race everyone was invited to enjoy a DJ sponsored by Weems and Stephen's Equine Hospital (Aubrey, TX).



The forum was sponsored by the following: Select Breeders Service, Inc., Breeders Choice, Minitube of America, Plastilite Corporation, XY, Inc., Pavesafe (a division of Dodge-Regupol, Inc.), MVE, Inc., Intervet, Inc. Door prizes were donated from Select Breeders Southwest, Inc., Breeders Choice, Minitube of America, Plastilite Corporation and MVE, Inc. The prizes were given away during the dinner festivities.

Planning for the Third Annual Texas Breeders Forum in 2002 has begun. A pre-Breeding Season 2002 workshop is also in the works. To get on the mailing list for the upcoming events please contact Select Breeders Southwest, Inc. at (940) 365-2467 or E-mail at sbsw1@airmail.net Information will be sent to you as soon as it is available. ■

## Low Dose Insemination

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Generally, mares are inseminated with 500 million progressively motile fresh sperm, 1 billion total sperm that have been cooled, and 800 million to 1 billion spermatozoa that have been frozen and thawed. These large sperm numbers limit the number of mares that can be bred. Recent studies have shown that much lower sperm numbers can be used if insemination is done deep into the uterine horn or onto the uterotubal junction (low-dose insemination). The possible advantages of low-dose insemination include: 1) increase in number of mares bred per stallion, 2) use of stallions with poor semen quality, 3) frozen semen of limited supply, 4) use of sexed semen, and 5) reduced post-breeding endometritis.

Several methods have been used to inseminate low numbers of spermatozoa. These include surgical, oviductal insemination, commonly called gamete intrafallopian tube transfer (GIFT). With this method, a small number of spermatozoa are placed directly in the oviduct with oocytes (eggs). GIFT is a potentially valuable technique to produce pregnancies from subfertile stallions, frozen semen of low fertility and possibly sexed sperm.

Other means of inseminating with low numbers of sperm include deep uterine insemination, or through the use of a videoendoscope. With deep uterine insemination, a catheter is placed through the cervix into the body of the uterus. A hand is then placed within the rectum and the catheter is directed up the uterine horn to the very tip of the uterine horn. Placement of the catheter can be verified by ultrasound examination or by rectally palpating the uterotubal junction. Several additional studies have been conducted using the videoendoscope to inseminate low numbers of spermatozoa onto the uterotubal junction. Pregnancy rates for mares inseminated with 1, 5 or 10 million spermatozoa were 64, 75 and 60%, respectively (Morris et al., 2000).

In a series of studies on the fertility of sex-sorted spermatozoa, a videoendoscope was used to inseminate mares with either 5 million non-sorted sperm or 5 million spermatozoa sorted for X and Y chromosomes. It is extremely encouraging that

AT PETERSON & SMITH EQUINE Practice we have progressed from the antiquated theory that a mare needs to be palpated 3 or 4 times daily when breeding with frozen semen.

### Kid Clue - *continued from page 1*

respond to the ovulatory agent).

MARES BRED TO KID CLUE were embryo donor mares as well as normal broodmares. The following is a presentation of our results:

#### Breeding Results: Kid Clue 2001

*Total cycles bred: 157*

*% of cycles resulting in a pregnancy or embryo recovery = 46% per cycle*

*% of donor mares that resulted in successful embryo transfer pregnancies = 82%*

*% of broodmares resulting in a pregnancy following insemination = 86%*

These results include all mares and all cycles; independent of age, (2 through 22 years) and reproductive history. Even if the mare became anovulatory, the cycles were included in the data. Certainly there were a number of very tough mares, as with any clinical situation.

ALSO OF INTEREST is how predictably these mares ovulated with this protocol: 75% ovulated by the second insemination and 93% ovulated by the third insemination. This management allowed us to conserve precious semen while achieving a good pregnancy rate. It also enabled us to manage these mares in a time efficient and cost efficient manner. ■

pregnancy rates in the range of 40 to 50% can be obtained with insemination of 5 million spermatozoa. This is particularly true when the spermatozoa have been damaged by freezing and thawing or by sorting for X and Y chromosomes with a flow cytometer.

It is obvious that placing the sperm on the uterotubal junction allows one to decrease the number of sperm required per pregnancy. In a clinical study conducted in Brazil, 166 mares were inseminated with 100 to 150 progressively

motile frozen-thawed spermatozoa. This number is about one-third of the normal dose of frozen-thawed semen. This group of researchers obtained 95 pregnancies from this clinical trial (57%).

The technique of low-dose insemination with a videoendoscope is an expensive technique and should be utilized only in special situations where semen is of poor quality, in limited supply or sexed semen is being used. ■

