contents

equine semen & embryo freezing, storage & distribuiton

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Select Breeders Services - Worldwide Semen Freezing & Distribution

WINTER 2003

FOUNDATIONS

Technology & Service Strengthening the Equine Breeding Industry

Don't Be Fooled

Frozen Semen Myths & Misconceptions..Part II

In the last issue of Foundations, we introduced the subject of the myths and misconceptions stallion owners often face when marketing frozen semen. These biases surface as resistance from mare owners or veterinarians who may have had a previous bad experience using frozen semen or myths that have been propagated over the years.

Two of the most frequently misunderstood issues were discussed:

- Breeding mares with frozen semen requires extensive "round the clock" veterinary examinations to achieve acceptable pregnancy rates.
- Frozen semen is more expensive to use than cooled transported semen.

In this issue, we will address the following four topics:

- Many mares have allergic reactions to frozen semen extenders.
- Frozen semen fertility is much lower than cooled semen fertility.
- Thawing and handling frozen semen is technically very difficult and therefore requires a veterinarian with a lot of previous experience using frozen semen.
- If semen from a particular stallion doesn't cool well then it will definitely not freeze well.

If you missed the summer issue of Foundations, the complete

continued on page 2

SBSW Unveils A New Mobile Laboratory

Select Breeders introduced the industry's first mobile freezing laboratory in 1994 to bring frozen semen technology to farms and veterinary clinics throughout the US. After so many years of hard work, it was time to retire the lab and construct a new one. In November 2002, Select Breeders Southwest took possession of a 26' custom designed mobile lab (pictured here), built by LaBoit Industries. This mobile lab is outfitted with state-of the-art equipment and will more readily service the many clients that demand and enjoy on-site service.

With the relatively recent acceptance of frozen semen by the American Quarter Horse Association, American Paint Horse Association, and the Appaloosa Horse Club, the new

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Misconceptions

continued from page 1

article can be found on our website, selectbreeders.com.

Myth III

Frozen semen fertility is much lower than cooled semen fertility.

Not all stallions produce sperm that can be frozen successfully and selection of stallions for use in commercial frozen semen breeding programs is essential. In general, per cycle pregnancy rates for mares bred with frozen semen are slightly lower (~10%) than for mares bred with cooled semen. However, seasonal pregnancy rates have been found to be similar. We compiled data from three commercial transported cooled semen programs in which semen from 16 stallions was used for insemination of 850 mares throughout North America by local veterinarians. During the 1999 and 2000 breeding seasons, first cycle and seasonal pregnancy rates of 59.4 and 74.7% were obtained. During that same period, first cycle and seasonal pregnancy rates of 51.3 and 75.6% were obtained following insemination of 876 mares with frozen semen from 106 different stallions processed by our laboratory and distributed through our commercial distribution program.

Myth IV

Many mares have allergic reactions to frozen semen extenders.

Practitioners and mare owners have reported that some mares inseminated with frozen semen exhibited a post-breeding endometritis, presumably in response to some component of the frozen semen extender. Because frozen semen extenders are different than other semen extenders in that they contain egg yolk and glycerol, it was thought that the mares were adversely reacting to one of these components. Recent studies have proven this not to be the case. It has been clearly demonstrated that all mares have an immediate inflammatory response to the deposition of sperm in the uterus. This occurs with natural mating and artificial insemination of fresh, cooled or frozen semen. The seminal plasma present in semen plays a role in mediating this inflammatory response and promotes uterine clearance. With frozen semen inseminations there appears to be a delayed clearance of this normal, sterile, inflammatory fluid in some mares. This is likely due to the fact that prior to freezing, a majority of the seminal plasma is removed from semen by centrifugation as a necessary step for successful cryopreservation. Delayed uterine clearance of post-mating induced inflammatory fluids is most prevalent in older mares that have large uteruses, with poor tone that do not contract well making it difficult to physically clear fluid. Older maiden mares may also have a problem with mechanical clearance of fluid due to cervical dysfunction. It is recommended that mares exhibiting clear fluid in the uterus post-insemination be treated with oxytocin to promote uterine contractions and aid mechanical clearance.

post-thaws

▼ 2002 Colt
Custom Crome Dually
By: Dualin Gun
Out of: Maid Red By Impressor

▲ 2002 Filly
Blondes Dual It Best
By: Dualin Gun
Dam: Skeeter Blondiebar
Breeder: Diana & Jessica Fleek, Commerce, TX

Myth V

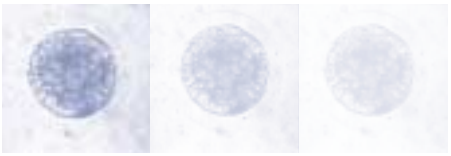
Thawing and handling frozen semen is technically very difficult and therefore requires a veterinarian with a lot of previous experience using frozen semen.

While it is true that equine spermatozoa are very sensitive to temperature change, and improper thawing and handling can damage the sperm, the actual process is very simple. Each shipment of frozen semen is accompanied by detailed thawing and handling instructions and arrives in a nitrogen container that will maintain the semen for several days after arrival. A water bath maintained at 37°C with an accurate thermometer, a pair of hemostats or tweezers to remove the straws and a sterile test tube or all-plastic syringe is all that is needed to thaw semen properly that has been frozen in 0.5 ml straws. For the past several years all protocols used by SBS for semen freezing utilize 0.5 ml straws. Semen frozen in large volume (4 or 5 ml) makrotubes requires the water bath temperature to be set at 50°C. When thawing at this temperature, the duration of time that the straw remains in the water bath is critical and therefore accurate timing (45 seconds) is essential. A veterinarian with a solid background in reproduction, artificial insemination and mare management is critical to the success of any breeding program using frozen semen.

Myth VI

If semen from a particular stallion doesn't cool well then it will definitely not freeze well.

A stallion whose semen does not cool well using standard procedures is not necessarily a poor candidate for semen freezing. There are many different factors that may negatively affect how well semen from a particular stallion will cool. Some of those factors do not adversely affect the ability of sperm to be successfully frozen. For example, stallions that typically ejaculate semen with a very low sperm concentration are difficult to cool successfully without concentrating the sperm via centrifugation prior to dilution with an extender. All ejaculates processed for freezing undergo centrifugation as a normal part of the protocol and may survive the process of freezing and thawing much better than cooling without centrifugation.



Multiple Embryo Transfers

E.L. Squires

Within the past year, several breed registries have changed their embryo transfer rule to allow unlimited registration of foals from embryo donors. This rule change has created controversy regarding the pro's and con's of such a procedure.

The advantage of registering multiple foals from a mare during a year is to give that mare a greater influence in the breed. Stallions have always been allowed to breed an unlimited number of mares in a year, whereas the mare's production has been limited. Embryo transfer will allow increased performance from some of the greatest mares in the breed. However, great mares are often not identified until late in their career. Multiple embryo transfer would allow one to catch up on production from these superior mares. Since embryo transfer is expensive, it is likely that the increased production will be from the very best mares in the breed. Furthermore, multiple embryo transfer would allow one to determine early in her life whether the mare is a good producer and what is the magic cross for that mare.

There are disadvantages that are either real or perceived by the breeder concerning multiple embryo transfer. The most common argument against multiple registration of foals is that we will flood the market and lower the price of foals. Other stated disadvantages include that this would drive the price of good mares higher, certain mares will dominate the breed and that lesser quality mares will go unbred. One of the more common complaints is that this procedure of getting multiple foals out of a mare in a given year is strictly for the rich. Another disadvantage is that repeated flushing of the mare's uterus may be harmful to the reproductive health of the mare.

Only time will allow us to tell whether or not acceptance of this technology helped or hindered the breed. It is my prediction that, although there is no restriction on the number of foals that can be produced, most breeders are satisfied with only a maximum of 2 to 3 foals per year. One commonly expressed fear is that a mare will produce 10 or 20 foals in a year's time and several hundred in a lifetime. This statement is usually based on the breeder's knowledge of what has happened in the cattle industry. However, based on our experience with Warmbloods, it is extremely difficult to obtain more than 3 to 5 foals from a given mare in a year's time. This requires that the mare be flushed nearly every cycle and that pregnancy rates are high upon transfer of the embryo.

post-thaw

Convoy by Contigo M
out of Plum Foxy (TB)
breeder: Sarah Lundy

Appaloosa Horse Club Approves Frozen Semen

Starting in 2003 The Appaloosa Horse Club (ApHC) will accept the registration of foals conceived via the use of frozen semen. As with cooled transported semen, stallion owners will be required to complete breeding reports to be filed with the ApHC and must apply for and receive a Transported Semen Stallion Permit from the ApHC prior to transporting any semen. All stallions participating in the program must have their DNA on file with the ApHC before a permit will be issued. The rule limits the use of frozen semen to the end of the calendar year of the death of a stallion and frozen semen cannot be used after a stallion has been gelded. All foals resulting from transported semen must be parentage verified by DNA genetic testing prior to registration.

The Appaloosa Horse Club, Inc., was organized in 1938 by a group of men determined to keep the famed Nez Perce war and hunting horses from slipping out of sight forever. Since then the growth of the Appaloosa Horse Club, Inc., has been rapid. The influence of the Appaloosa horse is spread around the globe with International Affiliates of the ApHC in most European countries as well as Australia and New Zealand.

spotlight

Dutch Warmblood
by Consul
Owner/Breeder
Iron Spring Farm

JUDGEMENT
Balancing Act

One of the primary benefits of frozen semen to owners of performance stallions is the ability to participate in the breeding season without interrupting a heavy show schedule. Judgement, owned by Iron Spring Farm and the first American bred NA/WPN approved stallion is an outstanding example of combining an active breeding season with stellar performance records. Judgement, by Consul, was the 1999 American Gold Cup winner, ranked 22nd in the world for FEI Show Jumping, a member of the 2002 US WEG jumping team, and most recently the winner of the $60,000 Budweiser Prix de Penn National at the Pennsylvania National Horse Show.

During the 2002 breeding season, Judgement's frozen semen was shipped to 16 mares, with a pregnancy rate of 82%. Throughout that same period, Judgement was vigorously campaigning with Beezie Madden at Spruce Meadows and the USET Selection Trials.

According to Mary Alice Malone, owner of Iron Spring Farm, "Without the availability of frozen semen and Select Breeders Service excellent distribution system, there is no way Judgement could breed so many mares and achieve the show results he enjoys. Frozen semen allows our breeders to schedule around their mares, rather than our stallion's availability. It is a great arrangement for us and our breeders."

SBS Affiliated Laboratories

Select Breeders Service, Inc.
Paul R. Loomis
Colora, Maryland
(410) 658-3328

Select Breeders Southwest, Inc.
Whit Byers
Aubrey, Texas
(940) 365-2467

Peterson & Smith Equine Reproduction Center
Dr. Phil Matthews
Summerfield, Florida
(352) 307-3001

Hagyard-Davidson-McGeeAssociates
Dr. John V. Steiner
Lexington, Kentucky
(859) 255-8741

Gumz Farms, LLC
Amy Gumz
North Judson, Indiana
(574) 896-3355

Pioneer Equine Hospital
Dr. Duncan Peters
Dr. Hilda Baisel
Oakdale, California
(209) 847-5951

Advanced Equine Reproduction
Solvang, California
(805) 688-5080

The Winner's Circle Equine Fertility Centre
Dr. Bill Swyers
Dr. Rick Beck
Hemet, California
(909) 929-4533

NEW!

SBS EUROPE

Studio Veterinario Cristella
Dr. Sandro Barbacini
Cremona, ITALY
(39) 0372 65101

SBS Northgermany
Dr. Kirsten Schwenzer
Bakum Lüsche GERMANY
(49) 05438/9585-60

Equine Services
Nicole Oberstein
Bad Saeckingen GERMANY
(49) 0 170 549 1127

SBS AUSTRALASIA
in partnership with
Goulburn Valley Equine Hospital
Dr. Angus McKinnon
Shepparton, Victoria, AUSTRALIA
(61) 03 58 299 566

European QH Market

The Quarter Horse is creating an entirely new horse market in many parts of Europe - attracting new people into what has always been a well established horse industry. Europeans are discovering the appeal of the Quarter Horse - ease of handling, smooth riding, and fun nature of Western horse shows. Additionally, the fascination with all things 'cowboy' is still quite popular throughout Europe.

For example, during the past few years Germany recorded a 43% increase (1997-2001) and Italy a 33% (1998-2001) in ownership of registered Quarter Horses. Although the base is relatively small compared to the US, the growth trend continues its upward spiral.

If anyone questions the incredible popularity of Quarter Horses in Europe, you only have to look at the attendance figures of Americana 2002. Now in its 11th year in Augsberg, Germany, this strictly Western style horse show recorded over 50,000 visitors during the eight day run. For the exhibitors, a total of $500,000 in prize money was distributed. European mare owners are eager to access some of the world famous Quarter Horse sires from America. In response to this need, Select Breeders Services was invited to present a series of seminars at Americana on the importation and use of frozen semen. SBS speakers included: Whit Byers, Select Breeders Southwest, Dr. Sandro Barbacini, Studio Veterinario Cristella, SBS Europe, Nicole Oberstein, Equine Services, Dr. Kirsten Schwenzer SBS Northgermany and Dr. Ed Squires, SBS consultant and Professor of Biomedical Sciences at Colorado State University.

from left: *Paul Loomis (SBS), Dr. Kirsten Schwenzer (SBS Northgermany), Francesca Bellingeri (SBSE), Nicole Oberstein (Equine Services), Kathy Byers (SBSW), Dr. Ed Squires, Whit Byers (SBSW), Dr. Sandro Barbacini (SBSE)*

news

NEW SBS Affiliate Laboratory

The Winner's Circle Equine Fertility Centre

39185 Diamond Valley Rd.
Hemet, California 92543
909-929-4533
Fax 909-929-2077
HOSDOK2@aol.com

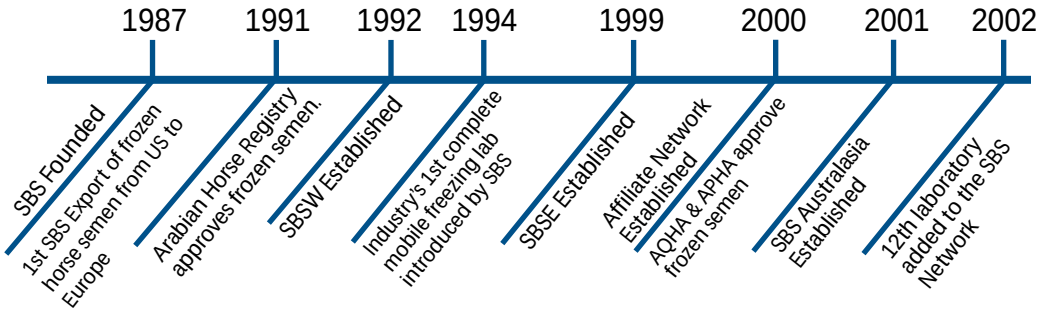
Committed to Professional Equine Medicine, Surgery, and Reproduction.

The newest SBS Affiliate, The Winners Circle Equine Fertility Centre, is strategically located in Hemet, CA less than one hour from San Diego, Los Angeles, Riverside, and San Bernardino. The clinic's practitioners, Dr. Rick Beck and Dr. Bill Swyers are both graduates of Colorado State University's School of Veterinary Medicine and each has a diverse and extensive background in equine reproduction. Their facility is specifically designed to provide the best possible reproductive care including a complete medical and surgical hospital, fully equipped reproduction laboratory, dry and wet mare care pens or stalls, grass pasture, a separate breeding and stallion barn, and oversized stallion stalls with large grass paddocks.

As part of the SBS Network, Winners Circle brings to the Southern California stallion owner the same professional semen freezing evaluations, freezing services for domestic and international use, frozen semen storage, and the ease and security of global distribution that are the hallmarks of the network.

For mare owners, Winners Circle provides high-risk pregnancy monitoring, donor mare management and an on-site recipient herd for embryo transfer and oocyte transfer, oocyte collection and maturation as well as Gamete Intra-Fallopian Transfer Procedures (GIFT).

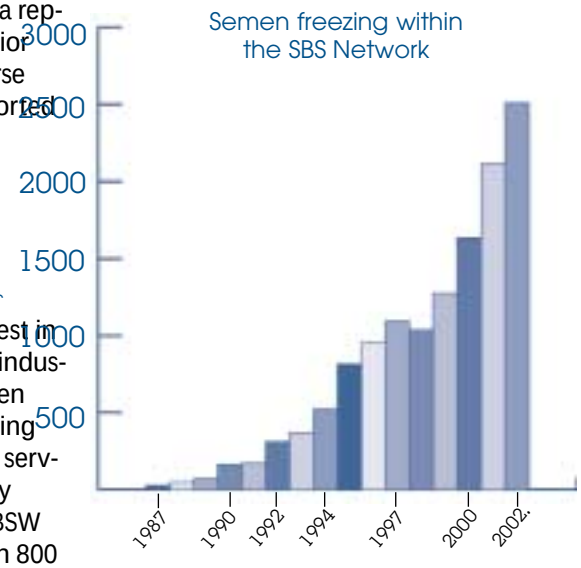
timeline



Managed Growth Serves our Clients

In 1987, Paul Loomis founded Select Breeders Service, Inc. with the goal of bringing frozen semen technology to horse breeders. Beginning with a single laboratory and the willingness to travel the United States to work with forward thinking breeders of Warmblood Sporthorses, SBS quickly developed a reputation for quality service and superior technology. In 1991, the Arabian Horse Registry approved the use of transported semen, both frozen and cooled. In 1992, SBS moved its main laboratory and offices to their present location on the grounds of Hilltop Farm in Colora, Maryland. Also, in 1992, Whit Byers and Paul Loomis co-founded Select Breeders Southwest in Aubrey, Texas. SBSW introduced the industry's first fully equipped mobile semen freezing laboratory in 1994 recognizing the need to provide on-site freezing services to stallion owners and veterinary clinics. By the mid 1990's, SBS and SBSW laboratories were freezing more than 800 ejaculates per year from Warmblood, Morgan, Standardbred, Saddlebred and Arabian stallions throughout the U.S.

Dr. Sandro Barbacini, from Parma in Northern Italy, began training with Paul Loomis in the SBS protocols for freezing semen in 1994 leading to the formation of Select Breeders Service-Europe in 1999. In the year 2000, SBS launched it's highly successful Affiliate Laboratory Program in partnership with leading veterinary clinics and reproductive management facilities bringing SBS semen freezing services to breeders throughout the United States and Europe . During that same year, Dr. Ed Squires, of Colorado State University joined SBS as a consultant and two of the world's largest breed registries, the American Quarter Horse and American Paint Horse approved the use of frozen semen. In 2002, SBS expanded its global reach with the formation of Select Breeders Service, Australasia in partnership with Dr. Angus McKinnon and the Goulburn Valley Equine Hospital. Today,



the SBS Network consists of eight laboratories in North America, three in Europe and one in Australia.

During the year 2002, American and European laboratories processed and froze more than 2500 ejaculates, an increase of 142% over the past 4 years. In many respects, this incredible growth record is due to our clients' belief in frozen semen technology and the benefits it provides. We appreciate your support and will work hard to continue to earn your business.

8th International Symposium on Equine Reproduction

E.L. Squires

Approximately 300 scientists and clinicians from 26 different countries gathered at Colorado State University for the 8th International Symposium on Equine Reproduction. These international symposia are held every 4 years as a venue for clinicians and scientists to present the latest information on equine reproduction. Presented in this article is a brief overview of information presented in the Stallion Section. Of the 150 abstracts presented either orally or as a poster, one-third of those concerned the stallion and/or semen. This is, by far, the largest number of papers presented on stallion reproduction at these international conferences. In the next issue of Foundations, a brief overview of the pregnant and non-pregnant mare sessions will be presented.

There were several presentations on the type of damage that occurs during cooling and freezing of sperm. Stallion sperm is exposed to both oxidative and osmotic stress during the freezing process. Seminal plasma, which is primarily removed during the freezing process, contains enzyme scavengers that protect against reactive oxygen species (ROS). It seems possible that, in the future, certain antioxidants may be added to semen extenders to prevent oxidative stress during freezing. Other workers demonstrated that cryopreservation increased DNA fragmentation and that the addition of α tocopherol tended to reduce DNA fragmentation. Some researchers have suggested that stallion sperm become precapacitated during the freezing process. This results in a shorter lifespan of sperm after thawing. Protein phosphorylation is an indicator of capacitation and measurement of tyrosine phosphorylation can be used as an indicator of the capacitation status of cryopreserved sperm.

Several reports were presented on alternative cryoprotectants for freezing stallion spermatozoa. It is encouraging that use of cryoprotectants, such as dimethyl formamide; at a concentration of 2 to 5% may be a suitable alternative for glycerol, particularly when freezing semen from certain stallions.

The role of seminal plasma in sperm survival during cryopreservation and when placed in the female reproductive tract was assessed in two separate studies. In the first study, sperm were diluted 1:1 with Kenney's extender and held for 2 hr at 5ºC prior to centrifugation. The supernatant was removed and sperm resuspended to a concentration of 200 million/ml in freezing extender only or freezing extender containing 50% seminal plasma. The ejaculate was then frozen and thawed. Adding seminal plasma improved both total and progressive motility. Fertility trials will be needed to determine whether this improvement in motility with additional seminal plasma results in increased fertility. The other study on seminal plasma was conducted to determine if seminal plasma protects sperm from binding to neutrophils, thus protecting the sperm against an inflammatory uterine environment. The addition of seminal plasma reduced binding of spermatozoa to neutrophils. A second experiment determined the effect of seminal plasma on pregnancy rates in mares that were inseminated in the presence of a post-breeding endometritis. Post-breeding endometritis was initiated by breeding the mare with either killed spermatozoa or semen extender. The mare was then rebred 12 hr after the initial insemination with 500 million progressively motile spermatozoa collected from a fertile stallion. The spermatozoa were centrifuged and extended in either 30 ml of semen extender or 30 ml of pooled seminal plasma. Only 1 out of 22 inseminations in the absence of seminal plasma resulted in a pregnancy, compared to 17 of 22 pregnancies when inseminations were done with seminal plasma added. The results of this study are in contrast with others that have reported that a second insemination during the cycle did not result in a reduction in fertility. In this study, mares bred with extended semen had essentially no seminal plasma compared with other studies where the level of seminal plasma may have been 5 to 10%. Further studies are needed to determine how much seminal plasma is beneficial in the inseminate.

When the amount of frozen-thawed semen is in limited supply, one possibility is to deposit the sperm at the uterotubal junction (UTJ). In one report, 10 million frozen-thawed spermatozoa were inseminated at the level of the UTJ and 33% of the mares became pregnant compared to 0% of the controls. Workers in California inseminated mares with 50 million progressively motile frozen-thawed spermatozoa at 24-hr intervals using a rectally guided UTJ insemination. They reported a 64% pregnancy rate compared to a 37% pregnancy rate for insemination of a standard dose of cooled semen. These researchers concluded that artificial insemination at the UTJ at a fixed time of 12 and 36 hr after hCG provided a practical approach.

Cryopreservation of epididymal sperm is utilized to preserve genetic material after injury or death of a valuable stallion. Three studies were presented on techniques for freezing epididymal sperm. In one study, testicle pairs were

review continued

8th International Symposium on Equine Reproduction

transported at 4ºC and, upon arrival 24 hr later, one testicle was processed immediately, while the other testicle was stored at 4ºC for either 48, 72 or 96 hr prior to collection of sperm. Epididymal sperm collected from the stallion testis stored at 4ºC retained adequate motility and viability for up to 96 hr. The objectives of the second study were also to determine the effects of storing the stallion's testes at 5ºC for 24 hr prior to collection of sperm for cryopreservation. Sperm from 6 of the 9 stallions was not adversely affected by storage in the testis at 5ºC for 24 hr compared with sperm processed immediately. However, progressive motility after freezing and thawing ranged from 0 to 27%. Addition of seminal plasma had no effect on the freezability of epididymal sperm. Workers in England evaluated the fertility of mares inseminated with epididymal sperm. When 200 million fresh epididymal sperm were deposited on the UTJ by videoendoscopic insemination, 9 of 20 conceived (45%). Insemination of frozen-thawed epididymal sperm (200 million) with UTJ insemination resulted in 9 of 51 becoming pregnant (18%) compared to 1 of 13 (8%) for conventional AI.

One very practical but quite important finding that came from this meeting was the observation that water-soluble lubricants used to prepare artificial vaginas may have a dramatic impact on sperm viability. In one report, the use of a water-soluble lubricant in the AV caused equine sperm cells in the ejaculate to be exposed to osmolalities as high as 800 mOsm. This resulted in a decrease in the percentage of motile spermatozoa. Contamination of semen with >10% of a water-soluble lubricant gel was detrimental to both fresh and cooled stored spermatozoa. One study evaluated the effects of commonly used lubricants, HR, Priority Care and KY Jelly, at levels of 0, 5 and 25%. Immediate exposure to KY resulted in a marked decrease in pH and reduced longevity of stallion spermatozoa. They concluded that Priority Care may be the best lubricant. However, it seems obvious that overuse of any water-soluble lubricant could result in a decrease in sperm viability.

calendar

Equine reproduction symposiums, Select Breeders Sponsored and Hosted events.

Management Strategies with Cooled & Frozen Semen

see dates below

Seminars with wet lab for veterinarians. Topics covered will include: collection,evaluation and packaging of cooled semen, factors affecting fertility of cooled & frozen semen, advantages & disadvantages of frozen semen, semen storage & handling, international transport, factors affecting pregnancy rates of frozen semen, mare management and breeding strategies for cooled & frozen semen.

Co-sponsored by Select Breeders Services and Intervet

February 1-2

Management Strategies with Cooled & Frozen Semen

Peterson & Smith Equine Reproduction Center
Ocala, Florida

To receive more information call
(352)307-3001

February 15-16

Management Strategies with Cooled & Frozen Semen

Winner's Circle Equine Fertility Centre
Hemet, California

To receive more information call
(909)929-4533

Summer 2003

4th Annual Texas Breeders Forum

Location TBA

To receive more information call
Select Breeders Southwest, Inc.
(940)365-2467

September 16-20

Society for Theriogenology Annual Conference & Symposium

Columbus, Ohio

Plenary Speaker: Fuller W. Bazer, PhD (Texas A&M) will be speaking on the future impact of reproduction and breeding

Annual Conference Sessions:
Food Animal, Small Animal, Equine

Symposia: Feline

www.therio.org

SBSW Satellite Collection Sites

mobile lab will be quite busy servicing farms specifically in Texas and Oklahoma.

continued from page 1

Additionally, to make scheduling easier for stallion owners in the two states, SBSW has created satellite representation at four veterinary clinics: Alpha Equine Breeding Center in Weatherford, TX, Kasper-Rigby Veterinary Assoc. in Magnolia, TX, Interstate Equine in Purcell, OK and Oklahoma City Equine Clinic in Oklahoma City, OK.

To schedule your stallion with Select Breeder Southwest at their main location in Aubrey, TX or to inquire about the mobile lab or the SBSW satellites, please call (970) 365-2467.