



# Foundations

Technology & Service Strengthening  
the Equine Breeding Industry

## Contents

### Quality is the Difference

Why breeders choose SBS first  
*page 1*

### Going Global

Frozen Semen Aids in  
International Marketing  
*page 2*

### Breeding Manager's Forum

Cholesterol-Loaded Cyclodextrins  
as a Means of Adding  
Cholesterol to Sperm  
*page 3*

### SBS Adds Two Affiliates

Two New SBS Affiliates  
in Ireland and Australia  
*page 6*

### Dietary Supplement for Stallions

*page 7*

### Select Breeders Services, Inc

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For more  
information  
about frozen  
semen and  
SBS locations  
visit:

[www.selectbreeders.com](http://www.selectbreeders.com)

### Search Stallions

[www.siredirectory.com](http://www.siredirectory.com)

## Quality Control is at the Core of the SBS Difference

### Paul Loomis

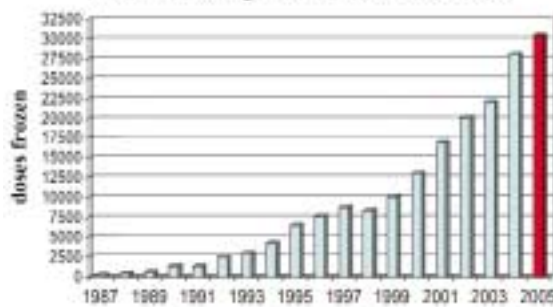
Founder & CEO,  
Select Breeders Service, Inc.

The SBS network of affiliates is the acknowledged leader in the field of cryopreservation of equine semen. Each year we freeze thousands of ejaculates equating to tens of thousands of doses. But what is behind those numbers? Why do we continue to grow steadily even in the face of increasing competition?

When SBS was founded, the company leadership made a commitment to quality of the finished product above all else. We wanted to put technology to use to help grow the

*continued on page 4*

Semen Freezing Within the SBS Network

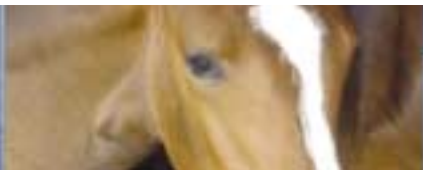


## Mobile Lab E-X-P-A-N-D-S SBS Maryland Capabilities

SBS Maryland has added a 22' mobile laboratory to better serve our off-site freezing customers. Because of our central location, the new lab allows us to more easily serve breeding farms and veterinary clinics throughout the Northeastern and Mid-Atlantic regions. The mobile lab is fully outfitted with all the equipment to collect, process and evaluate semen including a controlled rate cell freezer and Hamilton-Thorne computer assisted semen analysis system.

In addition to our USDA approved facility in Maryland, SBS Maryland has also developed relationships with several EU approved facilities from Maine to Virginia for freezing semen intended for export. The new mobile laboratory will enhance the service provided at these locations. For information on schedules and rates, please call us at 877-658-3328 or email to [info@selectbreeders.com](mailto:info@selectbreeders.com)





# Markets

## Going Global

### Frozen Semen aids in Global Marketing

The United States has long been the source for the best harness racing genetics and freezing semen from Standardbred stallions for export to Europe, Australia, and New Zealand is a thriving industry. SBS first exported Standardbred frozen semen in 1991 when semen from the stallion Harmonious, standing at Perretti Farm in NJ was frozen and exported to Europe. Select Breeders Service in Maryland alone annually exports over 2,000 doses of frozen standardbred semen to Italy, Sweden, Australia, and New Zealand. Because of the availability of frozen semen, breeders have the opportunity to breed to the world's leading sires regardless of where they are located.

Owners of the industry's most prominent stallions turn to SBS to preserve the genetics of their horses. For the past several years, the majority of the top placing horses at the Hambletonian, the oldest harness race in the US, have gone on to have their semen frozen and exported by SBS. The first and second placing horses for the 2005 race were sired by the same stallion, SJ's Photo. At the 2006 race, SBS client stallions Yankee Glide, Credit Winner and Muscles Yankee sired the top six finishing horses. SJ's Photo and Muscles Yankee are also among the

**>> SELECT BREEDERS SERVICE IN MARYLAND ALONE ANNUALLY EXPORTS OVER 2,000 DOSES OF FROZEN STANDARDBRED SEMEN TO ITALY, SWEDEN, AUSTRALIA AND NEW ZEALAND. <<**

leading money winning sires in the history of harness racing. We are proud that most of the leading standardbred farms in the US are SBS freezing clients.

**Frozen Semen aids in globalization of genetic pool.** The standardbred industry can serve as an excellent model for other breeds because of the globalization of the genetic pool. Stallion syndication agreements also reflect the international flavor of the business. Heretofore, syndicate members from Europe, Australia or New Zealand had to have their brood mares in the US

if they wanted to use their breeding shares. With the ease of shipping frozen semen abroad, they can take their shares in frozen semen and breed mares in their home

countries. Horses now racing in Sweden or Italy most probably have similar pedigrees to those racing in New Jersey or Ohio. For example, SBS began freezing and exporting SJ's Photo's semen to distributors in Europe in 1995. Today there are hun-



SJ's Photo

photo courtesy of United States Trotting Association

dreds of this stallion's offspring throughout Europe.

### 1991: First SBS export of Standardbred frozen semen to Europe.

Select Breeders Service's work in freezing semen for leading Standardbred sires provided the stimulus for our international expansion. After the initial export of Harmonious frozen semen in 1991, many other Standardbred farms used SBS to successfully freeze and export their stallions' semen. One stallion, Armbro Goal, standing at Castleton Farm in NJ, had had semen frozen for export by Paul Loomis/SBS for several years. The stallion was eventually sold to Orsi Mangelli Stallion Farm in Italy. When the freezing techniques used in Europe at the time didn't produce the same

results for Armbro Goal as those from SBS, Paul was asked to come to Italy to work with the Orsi Mangelli staff.

*continued on page 7*

photo courtesy of USTA



Muscles Yankee

## Cholesterol-Loaded Cyclodextrins as a Means of Adding Cholesterol to Sperm - Improving Post-Thaw Quality and Viability

Review by Dr. Ed Squires, Ph.D, ACT (hon)

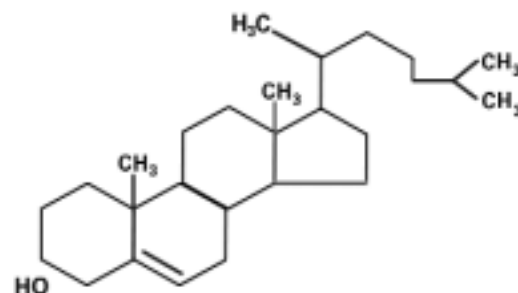
Sperm sensitivity to cold shock damage is determined by the membrane phospholipid composition and the membrane cholesterol to phospholipid ratio. Sperm with high cholesterol to phospholipid ratios (rabbit, human) are more resistant to cold shock than sperm having low cholesterol to phospholipid ratios (stallion, ram, bull). Cyclodextrins are cyclic oligosaccharides of glucose containing a hydrophobic core. Cyclodextrins can be loaded with cholesterol and used to deliver cholesterol to the sperm plasma membrane prior to freezing. Previous studies have shown that if stallion (Moore et al., 2005), bull or ram sperm are treated with cholesterol-loaded cyclodextrins (CLCs) before freezing, they exhibited greater survival after freezing than untreated sperm.

A study was conducted to determine if sperm concentration alters the beneficial effect of CLC on sperm survival after freezing and thawing (Moce and Graham, 2006). A second objective was to develop a procedure for adding CLCs to fresh bull ejaculates to improve cryosurvival. The results of this study demonstrated that adding 2 or 4 mg of CLC to sperm samples ranging in concentration from 120 to 2,000 x 10<sup>6</sup> sperm/ml resulted in a 17 to 28 percentage point increase in the number of live cells compared to controls. Incubating sperm with CLCs at 23 or 37°C before freezing resulted in similar survival. Adding CLCs to undiluted ejaculates (2 mg of CLC/120 x 10<sup>6</sup> sperm) resulted in greater percentages of live sperm compared with controls (62 vs 45%).

In a second study, the objectives were to determine the effects of specific cyclodextrins (2-hydroxypropyl and cholesterol-3-sulfate) on viability and capacitation of boar sperm following cold shock. The study demonstrated that incubation of boar sperm with 2-hydroxypropyl-β-cyclodextrin (HBCD) plus cholesterol improved post-cold shock viability. It completely inhibited tyrosine phosphorylation, which is an indication of capacitation. They concluded that preventing cholesterol loss from boar sperm during cold shock and freezing improved viability.

**Significance of these Studies**  
As stated previously, the cholesterol-phospholipid ratio in the plasma membrane of the sperm determines how well the sperm will survive freezing and thawing. Studies in all three species (bull, boar and stallion) have demonstrated that adding cholesterol to the plasma membrane using cyclodextrins has improved post-thaw semen quality and viability. Techniques have been developed that allow one to treat fresh ejaculates with cholesterol-loaded cyclodextrins prior to cryopreservation. It may be in the near future that these procedures are routinely used in freezing stallion sperm, particularly sperm from those stallions that routinely does not freeze well.

Currently we are conducting an experiment at Colorado State University to determine the fertility of mares bred with sperm that have been loaded with cholesterol prior to freezing. In order for fertilization to occur, cholesterol must come out of the sperm cell and allow the sperm



to be capacitated and, upon contacting the egg, be acrosome reacted. It will be interesting to determine if, once the sperm is loaded with cholesterol, will the cholesterol come out of the sperm easily, thus facilitating capacitation and acrosome reaction.

### References

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- Moce, E. and J.K. Graham. 2006. Cholesterol-loaded cyclodextrins added to fresh bull ejaculates improve sperm cryosurvival. *J. Anim. Sci.* 84:826-833.
- Moore, A.I., E.L. Squires and J.K. Graham. 2005. Adding cholesterol to the stallion sperm plasma membrane improves cryosurvival. *Cryobiology* 51:241-249.



For more Breeding Manager's Forum visit the Knowledge Library at [www.selectbreeders.com](http://www.selectbreeders.com)



# the SBS Difference

## SBS Quality Continued...

equine frozen semen AI industry in the most responsible manner possible. It was clear early on that one of the major factors limiting the application of frozen semen was inconsistent quality of the product being put on the market. Breeders and veterinarians experienced frustration with attempts to use expensive semen that in many cases was of very poor quality. We knew that in order for breeders to realize the full potential of frozen semen technology they would have to be convinced that by applying strict standards for quality, frozen semen could be produced to achieve results similar to cooled semen. We also knew that these results would have to be produced in an efficient and economical way. Quality became the cornerstone upon which we would build our business.

### Behind the scenes of the SBS Superior Quality Program.

When a client brings his stallion to an SBS facility for semen freezing, they are entrusting us with one of their most valuable assets. This is a responsibility that we take very seriously. Our promise to these customers is that we will use our 20 years of experience and all of the latest technology to produce the best possible frozen semen from their stallion. What we do behind the scenes is what makes us successful.

Because equine spermatozoa are very fragile and susceptible to damage from any number of factors, our QC program must deal with all phases of the process.



*Vapor pressure osmometer ensures all extenders are osmotically balanced*

Regardless of how sophisticated your cryopreservation procedures are, the quality of a thawed sample is directly dependent on the initial quality of the collected semen sample. In addition to the inherent semen quality from an individual stallion, the collected semen can also be influenced by many factors such as collection frequency, collection technique, number of mounts required for ejaculation, AV contamination, temperature shock, inappropriate AV, laboratory, and incubator or water bath temperatures), improperly calibrated equipment, etc. and many other often overlooked factors. All SBS labs routinely measure and maintain logs of numerous such details so that they are never factors that contribute to a poor result.

### Extenders are the most important factor for successful freezing outcome.

SBS cryopreservation extenders are proprietary formulations that have been developed over the last 20 years and have been used in conjunction with our freezing protocols to process literally tens of thousands of ejaculates of stallion semen. The cryopreservation media may be the single most important factor for success and that is why all extenders for North

American Labs are produced at our main laboratory in Maryland and shipped complete and frozen on dry ice to our affiliates as they are needed. Our SBSE facility in Italy produces all the extenders for our European network labs and extenders for



*extender production is centralized to ensure consistency*

Australasia are produced by a single laboratory in Australia. All extenders are made with the finest cell-culture grade reagents and ultra-pure (18 megOhm) sterile distilled water. Furthermore, each lot of extenders is tested and adjusted to provide pH and osmotic pressure within a narrow critical range or the batch is discarded.

### Complete, objective information for mare and stallion owners.

One of our founding principles is that all parties in frozen semen transactions should be treated fairly and that means providing detailed and accurate information concerning semen quality.

When a mare owner or their veterinarian receives a shipment of frozen semen from SBS, they also receive detailed instructions on use of the semen for maximum success and a Frozen Semen Transaction Report that



*(CASA) Computer assisted sperm analysis provides objective & accurate post-thaw motility data*

details the expected quantity and quality of spermatozoa in the frozen semen doses. Veterinarians and mare owners have come to rely on the open, objective and detailed information provided when semen is shipped from an SBS lab. Our strict post-thaw evaluation protocols are second to none and



involve thawing two "test straws" from every single ejaculate frozen. The thawed semen is cultured for potential mare pathogens and counted using a hemacytometer or our new fluorescent cell counting device (see photo) to verify sperm numbers. An aliquot of the thawed semen is diluted in extender, incubated for 30 minutes at 37°C to provide a sort of "stress test" and then analyzed for critical motility characteristics using a sophisticated Hamilton-Thorne CEROS model computer assisted semen analyzer (CASA). Use of CASA provides detailed, accurate and most importantly objective measurements of sperm motility thus eliminating the bias or discrepancy between individual technicians that is inherent in subjective estimates of sperm motility.



*NucleoCounter for verification of final sperm concentration*

Although many of the SBS Affiliates have their own CASA systems (there are 10 throughout our worldwide network), our Quality Control program for affiliate labs requires that test freezes from all new stallions and a minimum of 20% of all samples are sent to the SBS central laboratory for evaluation as described above. In addition, all data sheets and freeze records are sent to the central lab so that we can evaluate stallion management, implementation of SBS Standard Operating Procedures and assist in troubleshooting difficult freezing stallions for each of the labs. Site visits, frequent communication, use of the resources on our company-wide intranet site and

annual technical meetings for all network affiliates round out the Affiliate Quality Control Program.

### SBS storage techniques protect our clients' valuable inventories.

Properly stored frozen semen can maintain viability for many decades and perhaps longer (see *How Long Does Frozen Semen Last?*, *Foundations-Winter 05-06*) but improperly stored frozen semen will deteriorate and result in lower quality and fertility. The most troubling aspect of this reality is that there is no way to determine if straws of frozen semen have been improperly stored or handled without thawing and assessing semen quality. And in some cases the damage may be to structures in the sperm membrane that are very difficult or impossible to detect.

SBS maintains the largest inventory of equine semen in North America and possibly in the world. We take this responsibility very seriously. All semen is stored immersed in liquid nitrogen containers that are measured weekly and logged and filled to maintain semen quality at maximum level. Monitoring nitrogen consumption in liquid storage containers allows us to detect when a container may be losing vacuum and failing well in advance so that the semen can be transferred to another container before valuable inventory is lost due to container failure.

In the end everything we do is geared towards success for our clients. Frozen semen is a wonderful, efficient technology that removes geographical and time limitations when breeding superior horses but realizing this potential requires deliberate, steadfast adherence to quality standards. SBS has led the way in establishing these standards and will continue to raise the bar for the benefit of our clients as we head into our next 20 years.

### Our Affiliate Quality Control program guarantees consistent results.

Although many of the SBS Affiliates have their own CASA systems (there are 10 throughout our worldwide network), our Quality Control program for affiliate labs requires that test freezes from all new stallions and a minimum of 20% of all samples are sent to the SBS central laboratory for evaluation as described above. In addition, all data sheets and freeze records are sent to the central lab so that we can evaluate stallion management, implementation of SBS Standard Operating Procedures and assist in troubleshooting difficult freezing stallions for each of the labs. Site visits, frequent communication, use of the resources on our company-wide intranet site and



The semen used in this breeding was frozen in September 1994 and stored with Select Breeders at our Maryland storage facility for 11 years. The mare conceived on the first cycle.

**TLF Valimar**  
**06 American Warmblood Colt**  
**by Valinor** (Westphalian)(g)  
o/o Thallene Mist (Tb)

**Breeder:**  
Mary Barrett  
Twin Lights Farm

**Freezing/Storage Lab**  
Select Breeders Services  
Maryland



# News

## SBS WELCOMES...

New affiliates in Ireland & Australia



For more information about our 16 Network Locations visit us online at: [www.selectbreeders.com](http://www.selectbreeders.com)



### EQUINE VETERINARY SERVICES



>> For more information:

Dr. Andrea Carli  
Dr. Stefania Bucca

353 087 2708743 (p)  
[xyequine05@hotmail.com](mailto:xyequine05@hotmail.com)

**Straffan, County Kildare  
Republic of Ireland**

Drs. Stefania Bucca and Andrea Carli founded XY Equine Veterinary Services in November, 2005. With 30 years of experience in the reproductive field between them, the doctors' goal is to provide high quality equine semen freezing and related services to the Irish breeding industry. XY Equine is based at Knightfield Stud, Straffan, County Kildare and is centrally located in the Republic of Ireland about 1 hour from the Dublin airport.

XY Equine Veterinary Services is one of only a very few assisted reproductive clinics managed by highly trained veterinarians in the country. The reproductive services offered include: breeding soundness evaluations, management of sub-fertile mares, mare management for natural breeding, AI with fresh cooled and frozen semen, deep horn hysteroscopy insemination techniques, embryo transfer, fetal gender determination, and assessment of feto-placental well-being & management of high-risk pregnancies. As an SBSE affiliate laboratory, XY Equine is EU approved for export and offers equine semen freezing, storage and distribution as well as semen collection for cooled transport.

## Tamworth Equine Veterinary Centre

Tamworth, New South Wales - Australia

Tamworth Equine Veterinary Centre is located in Tamworth, New South Wales, in the center of Australia's quarter horse industry. The facility is located on 30 acres and is able to accommodate several stallions at any one time.

Tamworth Equine has been specifically designed to be both a veterinary and breeding facility. Reproductive services include residential stallion breeding, artificial insemination with fresh chilled and frozen semen, stallion collection for shipping around the country, stallion training and semen assessment, semen freezing, embryo transfer, and on-farm breeding management. General veterinary services are provided to the region and referrals come in from practices throughout the northern part of the state.

The principal, Stuart Keller, has been a veterinarian for 26 years; the last 16 in Tamworth. He has a specific interest in equine surgery and stallion reproduction. Dr. Keller is the honorary veterinarian to the Australian Quarter Horse Association and the



>> For more information

Dr. Stuart Keller

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[kelvet@bigpond.com](mailto:kelvet@bigpond.com)

National Cutting Horse Association. The permanent staff includes associate veterinarian Dr. Timothy Hill, Libby Keller, office manager, and Stuart's wife of 26 years; Kim Parkins, secretary; equine nurses and lab technicians Kate Duncan and Sophie McGarry; and Jodie Hansford, secretary/nurse.



## Global Marketing

*continued from p. 2*

Coincidentally, Dr. Sandro Barbacini, an Italian veterinarian specializing in reproduction, had been coming to the US for several years to train with Paul. Because Paul had trained Dr. Barbacini and knew he could produce results similar to those

obtained in the US, a partnership was established to freeze semen for Orsi Mangelli's stallions. The partnership with Dr. Barbacini

photo courtesy of USTA



Credit Winner

eventually grew into Select Breeders Service Europe. SBSE affiliate labs are located in Germany (North and South), Holland, Sweden, Ireland, as well as the original affiliate, SBSItalia. Each lab freezes using the same commitment to quality standards as the rest in the affiliate network.

### A true live foal guarantee demands quality frozen semen

The quality of the SBS produced frozen semen is the cornerstone of the relationship with the Standardbred owners. This industry has a true live foal guarantee. In fact, most stallion owners are only paid their breeding fee when the foals are born. In short, if the frozen semen they are using on their mares doesn't produce a pregnancy, they don't get paid. We are proud that so many of the Standardbred farms throughout the world turn to SBS affiliates each year for their freezing needs. It is those continuing relationships that reinforce our determination to be the first freezing service stallion owners call when they need the best quality frozen semen.

## New Dietary Supplement for Stallions

Recent research in stallion reproduction has demonstrated the beneficial effects of omega-3 fatty acids for enhancing reproductive performance in stallions. Bioniche Animal Health has introduced a new supplement called Magnitude. The product's stated goal is to improve stallion fertility by supplying essential long-chain polyunsaturated omega-3 fatty acids. Omega-3 fatty acids, docosahexaenoic acid (DHA) are key during spermatogenesis. DHA is a major component of spermatozoa's



plasma membrane and its deficit is linked to impaired fertility. Magnitude provides a marine based source of omega-3 fatty acids metabolically accessible for stallions. Recent studies at Colorado State University, University of Arizona and Texas A&M have linked Magnitude to increases in:

- sperm concentration
- daily sperm output

- the percentage of progressively motile sperm
- the percentage of morphologically normal sperm
- retention of cooled sperm motility up to 48 hours

The marine based source of omega-3 fatty acids used in Magnitude provides the accessibility without the storage and palatability problems associated with

fish oil. Plant based omega-3 fatty acids such as flax seed do not supply DHA.

Magnitude is sold in 20 lb containers which equates to a 26 day supply.

For more information or to order Magnitude please contact your nearest SBS Affiliate or call 877-658-3328 email [info@selectbreeders.com](mailto:info@selectbreeders.com).

# MOSCOW

The 17th Russian Equine Veterinary Association Conference was held during the Equiros Horse Exhibition in Moscow, August 16th-19th.



Attended by more than 100 veterinarians

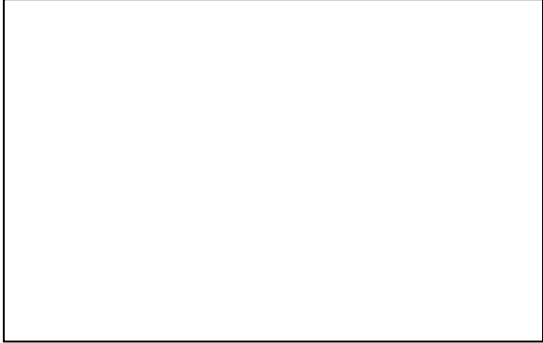
SBS was represented by Dr. Denis Necchi from SBS Italia. Dr. Necchi presented two topics: "Artificial Insemination in the Horse" and "Management of the Mare for Frozen Semen". Both topics featured a talk and practical demonstration and were well received.

# 2000<sup>th</sup>

## ..... 2000th Stallion Added to SBS Database

Every stallion frozen and or stored by SBS Network Laboratories is given a unique identification number to differentiate that horse's inventory of semen from any other. That information is entered into a database shared by all of the affiliates so if a horse is frozen by two different SBS affiliates, the same identification number is maintained. It is just one of the many ways we safeguard the inventory entrusted to us.

In April, 2006, we reached a significant milestone when the 2000th stallion was added to the database of client stallions. A Standardbred trotter named KILIMANJAROMONTANA, was frozen at [Gumz Farms, Inc.](#) in North Judson, Indiana for export to Europe.



Search for Stallions at [www.siredirectory.com](http://www.siredirectory.com)

**SBS Post-Thaw  
2006 Foal**  
Norwegian Fjord  
Stallion Owner  
Green Valley Farm  
Galena, IL  
Freezing/Storage Lab  
Select Breeders Southwest



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