

The international AI news from Minitube

# SpermNotes®

Special Edition: Spectrum Freezing Media

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# Innovative solutions for your success

Minitube's Research & Development is continually focused on promoting innovation. Today, we are proud to present a completely new concept in the field of equine semen preservation: We now offer a complete range of extenders for individual freezing protocols for stallion ejaculates that will revolutionise your processes and results.

The knowledge and experience gained over 30 years by Select Breeders Services (SBS) can now be passed on to equine veterinarians and breeders worldwide through Minitube.

By means of a Test Freeze Kit, the best freezing extender can be chosen for each individual stallion. Thus, more ejaculates will be frozen successfully and a higher quality of frozen semen will be achieved.

With this issue of our SpermNotes we want to introduce you to the world of the SBS CryoSystem. Further information on Minitube's product range and services can be retrieved at any time on our website [www.minitube.com](http://www.minitube.com).



Figure 1: Test Freeze Kit

## Imprint

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# Minitube and Select Breeders Services cooperation

Christa Simmet, DVM, Minitube



In 2014, Select Breeders Service, Inc (SBS), headquartered in Chesapeake City, Maryland, USA and Minitube International AG of Tiefenbach, Germany have signed a strategic collaboration agreement that aligned the world's largest provider of equine frozen semen services with the global innovation leader and provider of products and technologies for animal reproduction.

The agreement focuses on research and product development for equine reproduction. "I have long felt that a strategic partnership with a product development and manufacturing company such as Minitube would allow SBS to apply our 30 years of experience in equine reproduction to the improvement of products and procedures for the equine artificial insemination (AI) industry", said Paul Loomis, Founder and CEO of SBS. With its global network of 24 affiliated laboratories, SBS is well positioned to contribute to the development and testing of new products and procedures for equine reproduction produced from this collaboration. SBS is committed to the advancement of the science of equine reproduction and the responsible development of the frozen semen industry. This collaboration will go a long way towards advancing that goal.

Minitube has been pioneering a complete line of assisted reproduction products and services over the last 47 years and is serving clients worldwide in agricultural, sport and companion animal breeding as well as veterinary, medical and research communities. Providing products of uncompromising quality and safety, together with the commitment to continuously develop

new technologies and maintain company-owned manufacturing for proprietary products, comprise the cornerstone of our business approach.

In line with this long-term strategy, we at Minitube see the collaboration with SBS as an ideal platform for dedicated product and service development to provide integrated and comprehensive solutions for customers in the equine AI field. This collaboration now peaks in the launch of Spectrum extenders exclusively distributed by Minitube in markets outside the US and Australia that will be presented in this special issue of our SpermNotes.

The individual approach of the Spectrum extenders will contribute to the options of the equine frozen semen industry. Minitube is proud to make this new approach possible.



Figure 2: Combined knowledge, combined logo



# The SBS CryoSystem

Paul R. Loomis, Select Breeders Services



Damage from freezing and thawing can be attributed to destabilization of sperm membranes as cells move to and from storage temperatures (thermal stress) and osmotic stresses created during freezing and thawing.

Penetrating cryoprotective agents (CPA's) such as glycerol, DMSO, ethylene glycol and amides are added to semen freezing extenders to minimize the damaging effects of high solute concentrations and intracellular ice formation. The most common CPA in equine semen extenders is glycerol which has a permeability across the plasma membrane much lower than water, therefore glycerol moves into the cell after dilution in freezing extender at a much slower rate than water moves out leading to further dehydration of the cell during cooling/freezing. Conversely, when frozen sperm are thawed and placed in an environment free of glycerol (non-glycerol containing diluent for evaluation or mare uterine fluids after insemination), water in the environment moves into the cell much faster than glycerol diffuses out leading to rapid cell volume increases and disruption of cell membranes.

Lipid and protein sources such as egg yolk and milk are also common ingredients of semen extenders for their membrane stabilizing and antioxidant properties. Another major component of extenders are sugars such as glucose, lactose, raffinose, etc. which provide an energy source as well as act as non-penetrating CPA's.

Species differ in the susceptibility of their sperm to damage due to cold shock and cryopreservation. These species specific differences are thought to be related to the biochemical structure of the plasma membranes, specifically the cholesterol:phospholipid ratios, fatty acid content and membrane fluidity.

In addition to this species specific variability, a well-documented inherent variation exists between individual males of many species in the ability of their sperm to withstand the stresses associated with freezing and thawing (cryotolerance). This male to male variation is especially evident in stallions. In dairy cattle, bulls have been selected by the AI industry for more than 50 years based on the ability of their sperm to withstand the stresses of standard cryopreservation protocols. This selection has led to an increasingly uniform and positive response to cryopreservation. Studies on membrane fluidity and osmotic stress tolerance have demonstrated that bull sperm have a much greater tolerance for exposure to hypertonic conditions than stallion sperm and that there was a 3-fold greater variance in osmotic stress tolerance between individual stallions than between individual bulls. Studies

with boar sperm and human sperm have also revealed significant male to male variation in plasma membrane composition and some correlations have been found between cholesterol to phospholipid ratios, membrane fluidity, fatty acid content and response to cryopreservation. Further evidence for the relationship between membrane composition and cryosurvival comes from experiments with 4 different strains of mouse sperm that vary significantly in their cholesterol: phospholipid ratio. The percentage of motile sperm after thawing was directly correlated with the cholesterol:phospholipid ratio. The researchers were also able to dramatically improve cryosurvival in the low cholesterol strain by increasing the cholesterol content of the sperm membranes with cholesterol loaded cyclodextrins.

To date there is no single universal cryopreservation protocol that is optimum for semen from all stallions and use of a single protocol (extender, cooling rate, etc.) has led to the belief that stallions can be grouped into "good" and "bad" freezers based on post-thaw evaluation of semen frozen using a single common protocol. Based on studies conducted in the 1970's and 1980's, researchers concluded that as few as 20% of the stallions in the population produced semen that withstood the stress of freezing and thawing well and were considered good freezers. In their 1987 review of stallion semen cryopreservation, Amann and Pickett estimated that around 25% of stallions in the general population could achieve "acceptable" pregnancy rates with frozen semen while 30% would yield extremely low pregnancy rates with the remaining 45% falling between these extremes.

The SBS CryoSystem for freezing stallion semen is based on the belief that semen from a large percentage of stallions in the population can be frozen successfully if an effort is made to customize cryopreservation protocols to identify optimum conditions for each individual stallion.

## Our goals are to:

1. Produce the highest quality frozen semen from every individual stallion, not just accepting what appears to be adequate based on results from a single extender standard protocol and
2. Identify conditions for genetically desirable individual stallions deemed to be "poor freezers" that allows them to be included in commercial frozen semen breeding programs.

Excluding a champion performance stallion from a commercial frozen semen breeding program based on results from a single cryopreservation protocol is not acceptable if frozen semen is to be a significant tool in modern horse breeding.

# The SBS CryoSystem

The SBS Spectrum approach employs multiple protocols that are designed to determine the optimum procedure for maximum fertility of frozen semen from each individual stallion. Standard practice for all stallions is to collect several ejaculates to deplete extragonadal sperm reserves followed by 1-2 days sexual rest then perform 1 or 2 split-ejaculate test freeze procedures. The various extenders in the Spectrum series employ different sources and amounts of lipids, proteins, sugars and various penetrating and non-penetrating cryoprotectants designed to control damaging cell volume excursions during freezing and thawing.

## Test Freeze Data Analysis

Recently, we conducted an extensive retrospective study of data collected from stallions presented to two SBS laboratories in the United States that underwent split-ejaculate test freeze procedures during the years 1997 to 2016. The data included 1578 test freeze procedures on 1210 individual stallions of a wide variety of breeds and ages. Collected ejaculates were split into 2 to 4 fractions and frozen in 0.5 ml straws according to the standard cryopreservation protocols for each individual treatment using a controlled rate cell freezer. Pre-freeze and post-thaw motility was evaluated using computer assisted semen analysis (CASA). Test straws from each treatment were thawed, diluted to approximately 25 million/ml in an appropriate extender and incubated at 37°C for 30 minutes prior to CASA analysis. Progressive motility was defined as the percentage of sperm that exhibited an average path velocity (VAP) > 50 mic/second and a straightness ratio (STR) > 75%. An ejaculate was considered "acceptable" for commercial distribution if post-thaw motility was > 30%.

## Results

Overall 81% of ejaculates subjected to the test freeze procedure resulted in acceptable post-thaw motility of 30% or greater in one or more of the extender treatments tested.

If only one of the most common protocols had been used for these ejaculates only 64% of the freezes would have resulted in acceptable post-thaw progressive motility > 30%. Therefore, an additional 17% of stallions were frozen successfully when the split-ejaculate test freeze method was used to select an optimum protocol.

There was an increase of 10 percentage points in the percent post-thaw progressive motility on average when the optimum protocol was selected versus the single extender standard protocol. That represents a 33% increase in post-thaw motility.

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# Spectrum equine semen freezing extenders

Dr. Monika Esch, Minitube



Together, SBS and Minitube present you with the Spectrum equine semen freezing extenders based on formulations developed by and employed at SBS labs worldwide.

## Spectrum equine semen freezing extenders combine to form the SBS CryoSystem

The SBS CryoSystem consists of a range of freezing extenders which are paired with complementary freezing protocols. The SBS CryoSystem allows the clinician to identify the best freezing extender for each individual stallion.

Spectrum extenders are used to freeze stallion semen at SBS labs worldwide. Horse breeders have trusted the expertise of SBS for decades and veterinarians recognize and appreciate SBS labs as a source of high quality frozen semen.

Spectrum extenders feature various cryoprotectants and specific egg yolk or milk content that combine to protect sperm cells from damage caused by freezing and protect the fertility of the semen after thawing.

The unique SBS CryoSystem, consisting of Spectrum extenders and freezing protocols, has been tested on thousands of stallions. The results demonstrate that pregnancy rates can be achieved with frozen-thawed stallion semen which are comparable to those of cooled semen.

## Your benefits

- More ejaculates can be frozen successfully
- Overall higher quality of frozen semen
- Produce more commercial doses per ejaculate
- The Test Freeze Kit allows identification of the best semen freezing extender and protocol for each stallion
- The Test Freeze Kit contains all media and information needed to freeze split ejaculates
- While use of a programmable freezer will yield more consistent results, vapour freezing or programmable freezer can be used
- You can profit from the experience of SBS



Figure 3: The range of Spectrum extenders

All Spectrum extenders are produced in Minitube's GMP certified media production facility. Spectrum extenders are pH and osmolarity balanced and clarified. The chemicals are reagent grade, and bioassays of each batch are performed.

All Spectrum extenders are provided as ready to use solutions. There is no need to source individual components or to add antibiotics.

Spectrum extenders are supplied in convenient package sizes. Transport and storage is set at  $-20^{\circ}\text{C}$ .

# Spectrum equine semen freezing extenders

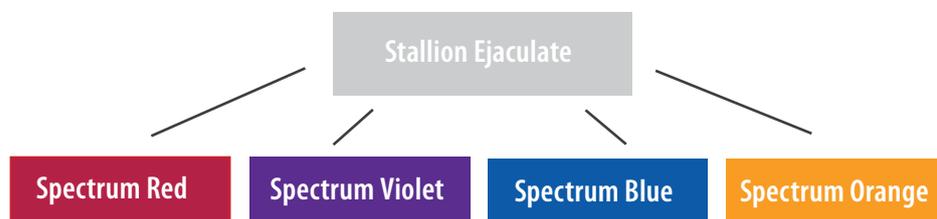
## Why is it important to have a range of freezing extenders available?

Unlike production livestock, stallions are generally not selected based on fertility testing. Within a given breeding population a high variability in the ability of sperm from individual stallions to survive cryopreservation is possible. This is likely due to differences in the lipid composition of sperm membranes. Use of a single extender or freezing protocol for all stallions may result in less than optimum results for many stallions.

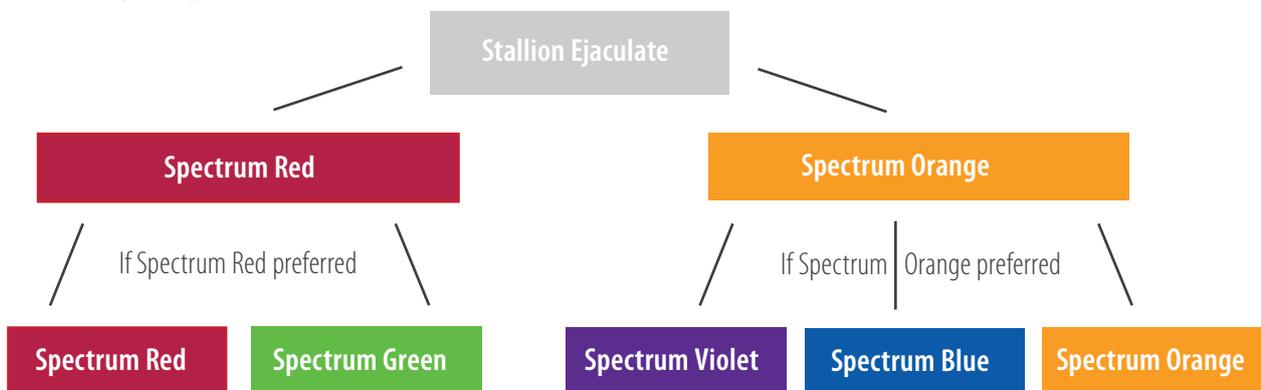
## How do we identify the best extender for each stallion?

Minitube offers a Test Freeze Kit which contains the main Spectrum extenders in smaller quantities and the centrifugation medium with CushionFluid for centrifugation. With the help of the split ejaculate trial outlined below, it is possible to find the best Spectrum extender for each stallion. Two approaches are recommended depending upon the amount of semen available.

1. Approach: If sufficient sperm are available (approx. 10 billion) - compare four of the extenders in a split-ejaculate test freeze



2. Approach: If < 10 billion sperm are available, split between Spectrum Red and Spectrum Orange first, then based upon this result perform a subsequent split as follows



## Learn more?

For more information, visit our website [www.minitube.com/Products/Equine/Semen-Extenders](http://www.minitube.com/Products/Equine/Semen-Extenders) and download our brochure on the SBS CryoSystem and Spectrum Extenders.



# Semen collection and optimizing quality of collected semen

Paul R. Loomis, Select Breeders Services

Because seminal plasma makes a poor culture media for sperm, the ideal type of ejaculate for semen preservation, either cooled or frozen, is one which has a low volume and high sperm concentration.

While total sperm output is relatively constant for stallions on a regular collection schedule, seminal volume and therefore sperm concentration in the semen is greatly affected by the amount of fluid contributions from the accessory sex glands. The amount of accessory sex gland fluid in the ejaculate is influenced by the degree of sexual stimulation prior to ejaculation. Ideally, the pre-collection stimulation should be just enough to cause the stallion to ejaculate on a single mount but not so much as to cause an excessive amount of accessory sex gland fluid. Efforts should be made to deflect the stallion's penis away from the artificial vagina to allow for the voiding of pre-ejaculatory secretions which for some stallions can contribute significant sperm-free fluid to the ejaculate volume.

In a large retrospective study of pre-freeze and post-thaw semen quality data from semen frozen by SBS labs Kalmer et al reported significant correlations between the number of mounts required for ejaculation and seminal characteristics. As the number of mounts required for ejaculation increased, seminal gel-free volume increased and both sperm concentration and initial

total and progressive motility decreased. **More importantly, as initial sperm concentration decreased, post-thaw total and progressive motility decreased ( $p < 0.001$ ).** Multiple mounts also lead to greater risk of sample contamination with pre-ejaculatory fluids and lubricant as well as debris and bacteria from the external genitalia and abdomen of the stallion. Significant loads of contaminating bacteria will adversely affect initial and post-thaw semen quality and so proper hygiene during collection and when handling semen is critical.

No. Mounts	No. of Ejaculates	Gel-free volume (ml)	Concentrations ( $10^6$ /ml)	Progressive motility (%)
1	9724	44.6 $\pm$ 0.7 <sup>a</sup>	247.5 $\pm$ 5.2 <sup>a</sup>	56.0 $\pm$ .11*
2	1914	52.8 $\pm$ 0.7 <sup>b</sup>	209.2 $\pm$ 5.7 <sup>b</sup>	55.4 $\pm$ .25
3	530	60.9 $\pm$ 0.9 <sup>c</sup>	182.0 $\pm$ 6.9 <sup>c</sup>	54.3 $\pm$ .49
4	169	70.0 $\pm$ 1.3 <sup>d</sup>	169.6 $\pm$ 9.4 <sup>c</sup>	53.6 $\pm$ .87

Figure 4: Effect of number of mounts on pre-freeze semen parameters N=761 stallions; 12,397 ejaculates

In a study on the prevalence and type of bacteria in extended chilled semen (Althouse), 66% of commercially prepared semen samples received by a group of clinics in the US were found to contain significant bacterial contaminants despite the antibiotics included in the commercial extenders. This illustrates the apparent lack of proper hygiene and sanitary protocols and antibiotic selection in the horse industry.

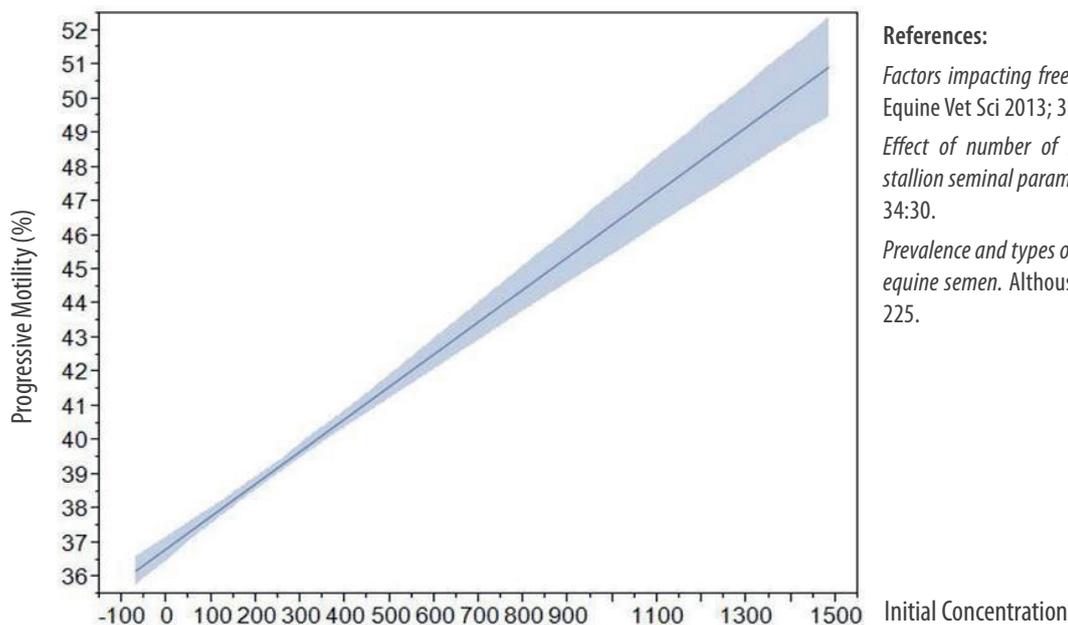


Figure 5: Effect of initial sperm concentration on post-thaw progressive motility

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# Egg Yolk in Stallion Sperm Extenders: Which came first – the egg or the extender?

Dr. Regina Oldenbourg, Minitube



The history of artificial insemination (AI) in horses traces back to the Russian military's need for horses in the late 19th century. And although the question posed above might just slightly overdo it, it is still interesting to follow up on it.

The first trials using media for semen dilution started in Russia only several years later (at the beginning of the 20th century). Presumably, the inclusion of egg yolk was part of those trial-and-error experiments, so it could easily even have been one of the first basic components of those early formulas. In fact, the first commercial yolk-containing semen extenders were developed in the 1940s. (The term 'extender' however was not coined until the year 1950 in order to improve the reputation of the idea – dilution was not meant to 'water down' semen portions, but helped 'extend' sperm viability.)

That research came just at the right time. Newly acquired knowledge on semen extenders, which were primarily developed for bulls, was picked up by American scientists advancing AI procedures in the horse (also in the 1940's). To this day, some components of media for both species remain the same - and egg yolk is one of the most prominent ones.

Curiously, the exact mechanisms of the protective properties of egg yolk on sperm cells remain unknown to this day, especially in stallion sperm. However, many studies suggest that low-density lipoproteins (LDL) are the main factor in cryoprotection of bull, ram, boar and dog sperm cells. Additionally, they enhance sperm viability and longevity during all stages of sperm processing (extension, storage, cooling/freezing).

Next to egg yolk, milk is also a popular ingredient of semen extenders. Both share membrane stabilizing and antioxidant properties. One shared characteristic of egg yolk and milk appears to be an interaction with harmful components present in seminal plasma, namely a family of lipid-binding proteins. In bulls, these proteins, called BSP (=Bovine Seminal Plasma Family of Proteins), have been widely studied. They are detrimental to sperm (cryo-) preservation by inducing phospholipid and cholesterol removal from the sperm membrane (thus destabilising it). Interestingly, BSPs interact with both low-density lipoproteins (LDL) present in egg yolk and casein micelles present in milk, thus preventing lipid removal from sperm membranes in a competitive manner. The sequestration of BSP proteins, e.g. by specific antibodies, might therefore be a notable method for improving stallion sperm storage if it could be made commercially available.

Recent studies have tried to find a more simple solution and have attempted to at least limit the inclusion of egg-yolk for freezing media for equine species to the supposedly active fraction (for example egg yolk plasma or liposomes composed of egg yolk phospholipid).

In summary, the inclusion of egg-yolk in semen extenders has an empirical base and the mode of action on a molecular and biochemical level remains elusive to the present day. However, the inclusion of it in semen extenders for stallion sperm still has undoubtedly great benefits and should be maintained as the development of alternatives is still evolving.

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Figure 6: Minitube extender production facility

# Frozen semen myths and misconceptions

Dr. Sandro Barbacini & Paul R. Loomis, Select Breeders Services

Stallion owners marketing frozen semen often are faced with resistance from mare owners or veterinarians who are biased against using frozen semen. These biases are usually the result of a previous bad experience using frozen semen or myths and misunderstandings that have been propagated over the years. This article will address those concerns and dispel some myths that may prevent mare owners from taking advantage of current frozen semen technology and stallion owners from maximizing their horse's marketability.

The following six issues are frequently misunderstood by breeders and veterinarians:

1. Breeding mares with frozen semen requires extensive "round the clock" veterinary examinations to achieve.
2. Frozen semen is more expensive to use than cooled transported semen.
3. Many mares have allergic reactions to frozen semen extenders.
4. Frozen semen fertility is much lower than cooled semen fertility.
5. Thawing and handling frozen semen is technically very difficult and therefore requires a veterinarian with a lot of previous experience using frozen semen.
6. If semen from a particular stallion doesn't cool well then it will definitely not freeze well.

## Myth # 1

*Breeding mares with frozen semen requires extensive "round the clock" veterinary examinations to achieve acceptable pregnancy rates.*

This is one of the most common misconceptions about the use of frozen semen. It is primarily a result of how frozen semen has been marketed over the years. Frozen semen sold "by the dose" with no guarantee has been prevalent in the Warmblood Sporthorse industry since the early 1980's. Mare owners would typically purchase 2 or 3 doses frozen semen with no guarantees and take it to their veterinarian to breed their mare. Knowing that the supply of semen was very expensive, the veterinarian tried to manage the mare so that only one dose of semen was used per heat cycle. Studies show that pregnancy rates are highest for frozen semen when mares are inseminated in the period of 12 hours before to 6 hours after ovulation. Although many fertility studies report very acceptable pregnancy rates for mares examined and inseminated only once daily during estrus up to the time of ovulation, it is quite clear that post-ovulation inseminations must be performed within 6 to 8 hours of ovulation. Since accurately predicting ovulation within 12 hours is very difficult, it is logical that a practitioner with only a single dose available would examine mares at 6 to 8 hour intervals in the periovulatory period, wait until ovulation is detected and inseminate a single dose at that time. There is also in vitro evidence

that sperm from some stallions that have been frozen and thawed may have a reduced ability to bind to mare's oviductal epithelium, which could reduce the lifespan of those sperm in the mare.

Recently, SBS developed and tested a simple and effective protocol for managing mares that are being inseminated with frozen semen. The new protocol involves a single daily examination until a 35 mm preovulatory follicle is detected, administration of an ovulation inducing agent (hCG or Ovuplant™), and insemination with two doses of semen; one each at 24 and 40 hours after administering the ovulating agent. Use of this protocol insures that viable sperm are available for fertilization in the mare's reproductive tract during the time of 12 hours before to 6 hours after ovulation for mares ovulating 18 to 52 hours after administration of hCG or Ovuplant™. Data from studies conducted at SBS Italia and Colorado State University as well as evidence from our own commercial distribution program indicate that similar pregnancy rates are achieved for mares bred using protocol and those bred with a single dose of semen within 6 hours of ovulation. Of course, this protocol requires that two doses per cycle are available to the practitioner. SBS encourages stallion owners to provide sufficient doses per cycle to utilize this protocol.

## Myth # 2

*Frozen semen is more expensive to use than cooled transported semen.*

In fact, the total cost for frozen semen to both mare and stallion owner is very similar. Costs for the stallion owner are primarily for semen production. Cooled semen production requires the building, equipping, and staffing of a collection and processing facility or contracting with a veterinarian or collection facility to provide the necessary services on an as-needed basis. Outside facilities usually charge 100-150 € to collect and process cooled semen. Since the useful life of cooled semen is 24-48 hours and most collections are for only one or two mares, the cost to the stallion owner can easily exceed 80 € per dose. Moreover, if the mares do not ovulate as predicted, a second collection for the same heat cycle may be required. Add to this the labor costs to trailer the stallion to a collection facility and the administrative costs to coordinate cooled semen requests, the overall semen production cost per mare bred can easily exceed 150 €. Depending on the number of sperm produced by any given stallion, frozen semen costs a total of 40-80 € per dose (including all labor and board at the collection facility). Typically, 2-3 doses are shipped per heat cycle, so the production costs per mare bred for frozen semen are similar to or even lower than those for cooled semen. Veterinary costs for mare management and insemination are comparable for cooled and frozen semen if there is more than one dose per cycle available and the protocol described in Myth # 1 is utilized. Mare owners are typically responsible for shipping/handling charges for cooled or frozen semen.

# Frozen semen myths and misconceptions

These charges are higher for frozen semen than for cooled semen, however the added benefits of using frozen semen outweigh the increased costs. With frozen semen, the shipment can be scheduled well in advance of the anticipated day of breeding, which eliminates concerns about last minute scheduling, shipment delays, or stallion availability when the mare is ready to be inseminated. Moreover, a single shipment of frozen semen can contain enough doses to inseminate a single mare through multiple cycles or multiple mares at a single location.

## Myth # 3

*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 (about 10%) than for mares bred with cooled semen. However, seasonal pregnancy rates have been found to be similar. The SBS laboratory based in Maryland, USA, 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 # 4

*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 postmating 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.

## Myth # 5

*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 # 6

*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.

# Minitube straw freezers: IceCube and Freezing Unit

## IceCube M and S: Compact computer controlled biological freezers for semen and embryos

The IceCube is a computer-operated freezer with intuitive interface for controlling and programming of multistage freezing curves. Color coded temperature curves are displayed during freezing and protocolled for later review. The operator is free to use the included standard freezing curves for semen and embryos, and to create customized freezing programs.

The IceCube is available in different sizes and versions to suit the users' specific needs. The freezer software and control is carried out by a connected PC, laptop or tablet computer and it can be equipped with a convenient touch display. Embryo and oocyte freezing can be performed in the IceCube with the autoseeding module and rack.

### Your benefits

- Programmed with standard multistage freezing curves
- Variable start temperature
- Data recorded for every single freezing cycle
- Different sizes (M + S), functions (+/- autoseeding for embryos) and technical setups (+/- touchscreen, with PC, laptop or tablet) to suit the users' specific needs
- Temperature curves as well as crystallisation inside straws can be observed during the freezing process
- Compact design
- Easy operation

**IceCube 14S** with connector kit for LN2 pressure container  
(Capacity: approx. 900 x 0.5 ml straws per freezing cycle)

**with 10" tablet**, no PC required [REF. : 16821/3000](#)

**with 12" touch screen monitor**,  
to connect to own PC or laptop [REF. : 16821/2000](#)

**without screen**,  
to connect to own PC or laptop [REF. : 16821/1000](#)



IceCube 14S with tablet-PC

**Larger alternative:** IceCube 14M (Capacity: approx. 2300 x 0.5 ml straws)

**IceCube 14M** [REF. : 16820/X000](#)

## Freezing Unit for straws

The "Freezing Unit" is a simple but very effective standardised vapour freezing system. The floating freeze rack includes a styrofoam chest, stainless steel inserts and a rack for 90 straws. The possibility of adjusting the height of the rack over the liquid nitrogen allows a wide range of applications.

When the freezing cycle is finished the straws are simply plunged into the liquid nitrogen. They can easily be transferred into a goblet and then be stored in a container.

**Freezing Unit** [REF. : 15043/0736](#)

