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THE FROZEN OPTION

In the cattle industry breeding by frozen semen has been the way to go for decades. The use of frozen semen by the horse industry has been slow to develop but is growing at a credible pace and potentially will replace cooled semen as the method of choice for shipped semen in the not so distant future. The use of frozen semen by Connemara breeders is in its infancy, but there is no reason why we can't take advantage of this technology like other sport horse breeders. To bring us up to speed and answer some commonly asked questions about frozen semen, American Connemara Magazine talked to Julie Skaife Ph.D. the Director of Quality Assurance for Select Breeders Services Inc., Colora, MD. With over 20 years of commitment to the frozen semen industry and 15 affiliated labs throughout the world the Select Breeders Services (SBS) network of affiliated laboratories is the acknowledged leader in the field of cryopreservation of equine semen. Select Breeders Services freezes >30,000 doses annually and sends out >1,800 shipments a year. Furthermore, SBS is setting the standards for frozen semen with a pioneering quality control program and commitment to incorporating the latest technology.

ACM: What is involved in freezing semen?

Skaife: If a stallion has not been collected recently we will begin with a series of clean out collections. This is because aged stored sperm in the epididymis and efferent ducts of the testes are often of poorer quality and do not respond as well to freezing. Depending on the individual stallion this could be 2 to 5 clean out collections.

When we freeze a stallion for the first time we do a test freeze comparing four different protocols; the differences between each treatment being in either the extender formulation or the rate of cooling. We select for subsequent freezing the protocol which gives the best results as determined by post-thaw motility. After the period of clean out collections we will have a good idea of the stallion's sperm quality and production and we can then estimate what his anticipated number of doses/ejaculate will be. The test freeze will give us an indication of his post-thaw motility and whether he will be a good candidate for breeding by frozen semen. Assuming he responds well to semen freezing we usually proceed with regular collection for freezing on a Mon, Wed, Fri schedule.

To process semen for freezing, the raw semen is diluted with a standard skim-milk glucose based extender and centrifuged to concentrate the sperm cells and remove seminal plasma. Seminal plasma contains factors that can be both protective and harmful to sperm. Primarily it is advantageous to remove seminal plasma when freezing semen. In the past clinicians would remove all of the seminal plasma, however, current research suggests it is beneficial to retain some, because seminal plasma is known to limit the inflammatory response of the uterus when presented with sperm cells. Following centrifugation the sperm are diluted into the freezing extender which has a complex formulation including sugars, salts, egg yolk, skim milk and a cryoprotectant, usually glycerol. The semen is loaded into straws and frozen in liquid nitrogen vapor. This can be accomplished by one of two methods; either by floating a rack of straws over liquid nitrogen in a Styrofoam box or by a specially designed programmable cell freezing machine. All SBS laboratories utilize programmable cell freezers. A programmable freezer offers the significant advantage of being able to reliably control the rate of cooling to an accuracy of 0.1 °C/min. This enables us to design defined and precise freezing protocols that are consistent and repeatable with every freeze. Freezing over vapor offers limited ability to adjust the rate of cooling and does not have the same repeatability. Once frozen the straws are then stored in tanks of liquid nitrogen at -196°C.

ACM: Do all stallions have semen that can be frozen?

Skaife: Not all stallions can be successfully frozen. Our experience within the SBS Affiliate Network indicates that approximately 75% of all stallions with normal initial semen quality produce sperm that are able to withstand the stresses of freezing and thawing, based on the industry standard for commercial distribution of >30% post-thaw progressive motility.

ACM: How long does frozen semen last?

Skaife: There have been studies that suggest frozen semen may last as long as 50,000 years without decay because it is kept at such cold temperatures. We can look to the bovine industry who have been using frozen semen successfully for the last 40-50 years and at SBS in Maryland we have some of the oldest frozen horse semen in the US, from the Olympic gold medal winning jumper stallion, Abdullah. We have semen >20yrs old from this stallion that we ship both domestically and

internationally every year and continue to get excellent fertility. However the key to maintaining frozen semen is conscientious monitoring of the liquid nitrogen level in storage tanks to ensure semen is consistently stored at the correct temperature.

ACM: What are the advantages of frozen semen?

Skaife: Frozen semen allows international distribution of genetics and is far more economical and convenient than shipping the horses themselves. This has valid application for the Connemara breed where in-breeding coefficients are high in certain countries.

Stallion owners prefer the convenience of frozen semen over the necessity of always being on call for cooled semen collections, especially if their stallion has a competitive career or experienced collection facilities are not available close to their home farm. Many stallion owners simply use frozen semen as an insurance policy in case their stallion dies or is injured such that he can't be collected for cooled semen.

Since the frozen semen is shipped in dry shippers that can maintain temperature for 2-3 weeks, mare owners like the advantage of being able to order the frozen semen ahead of schedule so it is on hand and readily available at the time of ovulation.

ACM: What are the disadvantages of frozen semen?

Skaife: In one of the few published trials, in which the fertility of fresh, cooled and frozen semen was evaluated from the same stallion, the fertility of cooled semen was less than fresh and the fertility of frozen semen was slightly less than cooled semen (approx. 10% lower than cooled semen). However, in other studies based on large numbers of inseminations, the fertility of cooled and frozen semen was similar and both were slightly less than fresh. In order to optimize success, breeding with frozen semen requires a certain level of expertise and additional mare management. However, using our timed insemination protocol (discussed below in the answer to, "How many doses should I ship per cycle and allocate to each mare for the season?") mares can be managed in a similar manner to how they would be when bred with cooled semen.

ACM: What fertility should I expect with frozen semen?

Skaife: The fertility of frozen semen is highly dependent upon the stallion. In general, if a stallion has sperm that survives the freezing and thawing process, fertility should be 40-50% pregnant after one cycle and 70-85% pregnancy rate after the breeding season. Although sperm motility is used as an indicator of relative cell health, fertilization is a complex process that requires numerous functional attributes of both sperm and egg. Therefore the true fertility of any frozen (or cooled semen) can only be determined by properly timed insemination of reproductively healthy mares.

ACM: How much semen should I freeze?

Skaife: Stallion owners new to frozen semen generally freeze about 50 doses, conservatively enough for 10-12 mares. Once they have a better indication of the use and demand for their frozen semen they can schedule subsequent freezing accordingly. The average stallions produces 8 doses of frozen semen per ejaculate, therefore six ejaculates collected for freezing would be required to produce 50 doses; when collected on a Monday, Wednesday, Friday schedule this will take two weeks. The number of doses produced per ejaculate depends on the total sperm production of the individual stallion and can range from 3 to >25 doses/ejaculate.

ACM: How should I sell my frozen semen?

Skaife: Frozen semen is typically sold one of two ways; either the semen is sold by the dose (typically without a guarantee) or it is sold as a breeding contract with similar provisions as a cooled semen contract. In our opinion, if you give a "live foal" or "return service" guarantee with cooled semen then you should also give the same guarantee with frozen semen. We feel that this is the fairest system for both the stallion owner and the mare owner.

ACM: Why do many stallion owners sell semen by the dose?

Skaife: Stallion owners who sell semen by the dose cite the following reasons:

1. They have no control over how the semen is handled and utilized therefore they do not want to be responsible if a mare owner and their vet do not manage the mare carefully and waste semen. They feel this is especially true if the semen is sent to a broker in another country and then sold to a mare owner.
2. They get paid up front and do not have to worry that mishandling or poor management will result in bad fertility.
3. They typically charge a fee per dose that is equal to one third to half the cost of a guaranteed breeding contract and this is typically well in excess of the cost of the production of that frozen semen.

The advantage here is completely with the stallion owner, the mare owner takes on all the risk at considerable expense.

ACM: Why do you recommend selling frozen semen as a part of a contract rather than by the dose?

Skaife:

1. Unlike bovine frozen semen which is sold by the dose as a commodity, the frozen semen from young stallions is not thoroughly fertility tested before being sold on the market. A dairy farmer who purchases a dose of frozen semen from a Holstein bull from a reputable AI or genetics company can be very confident of the fertility potential of that dose of semen when handled

properly and inseminated into a fertile cow. The reason for this is that dairy sires have been selectively bred for milk production AND fertility, more specifically fertility in an AI program with frozen semen. The range in fertility per cycle for commercial AI bulls is very small and only a few percentage points separate high fertility bulls from average and low fertility bulls. Furthermore, bulls with low fertility with frozen semen are not commercially viable and are culled from commercial production so their frozen semen never reaches the market. Fertility testing of a young dairy sire's frozen semen involves the insemination of thousands of cows to provide the estimate of fertility for that bull and the basis for which the decision is made to keep him or cull him from commercial production. Contrast this to the situation with horses. Even if one had the required number of mares available, stallions do not produce the number of AI doses per ejaculate required to practically or cost effectively fertility test their frozen semen. Stallions are not selected based on fertility, let alone how well their semen freezes. The best a mare owner can hope for is that the semen has been frozen by a reputable laboratory with proven protocols and that the frozen semen has undergone a thorough post-thaw evaluation and that any semen put on the commercial market meets strict quality standards. It is our position that the risk associated with breeding horses (by any means) should be shared by the stallion and mare owners.

2. Selling semen by the dose encourages mare owners to try and minimize the number of doses used (purchased) so that a pregnancy is achieved at the lowest cost. This approach will result in much higher veterinary costs for management of the insemination of that mare and may also result in lower fertility in an attempt to conserve doses of semen.
3. Frozen semen sold by the dose is often sold without any restrictions on the number of breeding certificates issued and may encourage a mare owner to split multiple straw doses for insemination of more than a single mare. This could lead to lower overall fertility from many stallions and contractual problems between mare and stallion owners.

ACM: How should I structure my breeding contract?

Skaife: We suggest that breeding contracts for frozen semen are structured so the costs associated with the production of the frozen semen are separated from the value associated with the genetics. The mare owner pays a non-refundable "booking fee" that is set at a level to cover the production costs for the frozen semen for that mare. The remaining balance, or stud fee, is for the genetics of the stallion, which are only realized if a pregnancy is achieved and a foal produced. The contract can be written to provide for "up to N doses for the

season" after which the mare owner has the option to purchase additional doses at a higher cost per dose if they want to continue trying to breed that mare or you may wish to offer them the opportunity to return the following season, in which case they would repeat the booking fee. The cost of the frozen semen shipment is then paid by the mare owner and in most cases this is cheaper for them than covering the cost of collection, processing and shipping of a cooled semen shipment.

To calculate your production costs of the frozen semen for each mare you divide the total cost of freezing the semen by the number of doses frozen - this gives you your cost per dose. Based on the number of doses you decide to allocate to each mare per season, you can then calculate your cost per mare.

ACM: How many doses should I ship per cycle and allocate to each mare for the season?

Skaife: There are two strategies commonly used to inseminate mares with frozen equine semen. If semen is in relatively short supply and only one dose of semen is available, mares generally are examined three to four times per day and then inseminated within 12hr prior to ovulation or within 6hr post ovulation. Frequent ultrasound examinations are difficult to accomplish unless the mare is sent to a veterinary clinic where a clinician is available. An alternative approach is to inseminate mares at a fixed time after induction of ovulation.

Select Breeders Services developed and tested a simple and effective protocol for managing mares that are being inseminated with frozen semen. The protocol involves a single daily examination until a 35mm preovulatory follicle is detected, administration of an ovulation agent (hCG or Deslorelin), and insemination with two doses of semen; one each at 24 and 40 hours after administering the ovulation inducing agent. Use of this protocol insures that viable sperm are available for fertilisation 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 Deslorelin. Data obtained from studies conducted in Italy and Colorado as well as evidence from our own commercial distribution program, indicate that similar pregnancy rates are achieved for mares bred using this protocol and those inseminated with a single dose of semen within six hours of ovulation.

Mare owners can achieve excellent results with frozen semen and minimize mare management costs if they are provided with at least 2 doses per cycle. Not every mare takes on the first cycle - this is true for natural service, cooled or frozen semen. A mare with good fertility, under competent management, should be able to conceive in 3 or less cycles. Therefore many stallion owners set aside 5 or 6 doses of frozen semen for each mare per season. This means there are 2 doses available for the first and second cycle and 1 or 2 doses for the third cycle. There are multiple options in structuring your contract and we have stallion owners that allow anywhere from 3 to 10 doses of frozen semen for the season. At SBS we ship semen on a per cycle basis, consequently

limiting the potential opportunity for frozen semen to be wasted or used inappropriately by the mare owner.

ACM: How is the frozen semen shipped?

Skaife: Frozen semen is shipped in vapor shippers, so called dry shippers. The basic concept of these shippers is that they absorb liquid nitrogen into their walls. The shipper is filled with liquid nitrogen and thereby “charged” before use. Once fully charged the liquid is poured out of the container and the shipper can maintain temperature for 2-3 weeks. The shipper can be sent as a non-hazardous material because there is no liquid nitrogen in the tank.

The distribution program should be an important consideration when looking at frozen semen providers. There is nothing more frustrating than investing your money in freezing semen to discover that your provider has only 1 or 2 dry shippers and when your mare owner calls for a shipment they are both checked out and it may be two weeks before they come back. We feel it is essential that the shipping process be as convenient, cost effective and as hassle free for the mare owner as possible.

ACM: What is involved in freezing semen for export?

Skaife: The stallion must be collected at a facility approved by the USDA for collection of semen for export. Most countries have very specific requirements for importation of frozen semen including pre/post collection testing for diseases such as CEM, EVA and EIA. Semen cannot be qualified after it is frozen, only at the time of freezing by doing the appropriate health testing. Processing of the export takes place on both sides of the transaction. The mare owner needs to apply to their department of agriculture for an import permit; this can take several weeks so it is best to ask the mare owner to make the application as soon as your breeding contract is finalized. The freezing center in the US then prepares a health certificate for the stallion and has it approved and endorsed by the USDA. Once the health certificates have been approved, the tank is sealed and on its way.

ACM: What should I be aware of when importing frozen semen?

Skaife: It is important to get as much information about the frozen semen as possible - have pregnancies resulted from this frozen semen, what is the pregnancy rate per cycle or per season, what is the post-thaw motility and how is that motility evaluated? What is the concentration of sperm and how many straws constitute a dose?

Each dose of semen should have at least 30% post-thaw progressive motility and contain a minimum of 200 million progressively motile sperm per dose. However, the evaluation of post-thaw motility can vary between clinicians. At SBS we test post-thaw motility by computer assisted sperm analysis (CASA) after 30mins incubation at 37°C. This is to provide a stress test to the frozen semen that may reveal latent damage to the sperm caused by the freezing process that may not be evident immediately after thawing. Some clinicians may only look at sperm motility immediately after thawing. This value may be higher than a sample evaluated 30 minutes later and does not give any indication of the longevity of the semen. Clinics that do not have a CASA system determine motility visually by looking down the microscope; this method is subject to observer bias and shows poor consistency of application and accuracy between technicians. If you have any concerns about the frozen semen we recommend that you have it analyzed by an independent lab before importation.

Originally from the Lake District in England, Julie has an undergraduate degree in Physiology from the University of Sheffield and an MSc in Equine Science from the University of Wales. She came to the US to study at UC Davis, graduating with a PhD in Comparative Pathology from the School of Veterinary Medicine in 2003. Her area of research specialization being the osmotic and oxidative stress associated with cryopreservation of equine and primate sperm, published under her maiden name, Baumber. Julie owns H.K.'s Sassy Pants an 8yo Connemara x TB mare by MGRM Brigadoon, they are successfully competing at Novice Level eventing and First Level dressage. She also owns a Morgan mare, Double M Eden Quest, a retired FEI driving pony. If you have further questions about frozen semen or Select Breeders Services, please feel free to contact Julie at (877) 658-3328 or julieskaife@selectbreeders.com.