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It Only Takes One... Right?

Evaluation of semen quality and its relation to fertility

Paul Loomis, Founder & CEO,
Select Breeders Service, Inc.

How many sperm does it take to get a mare pregnant?

1 billion?...500 million?... One? Actually, any one of those answers could be correct under certain conditions. The only way to really answer that question is... "it depends".

Fertilization is a complex process requiring that both the sperm and egg possess a myriad of functional attributes expressed at the right time and in the right place. A motile sperm is not necessarily a fertile sperm. So, how many sperm must be deposited in the mare for "acceptable" fertility? It would seem that this would be the logical basis for determining sperm numbers in an insemination dose for commercially distributed semen. To achieve the goals of both the mare and stallion owner it is necessary for each dose of semen to contain sufficient numbers of functionally competent sperm to maximize the probability of conception. The relationship between sperm number and fertility is expressed as a typical dose response

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Cutting Edge Technology

Ovum Pick Up and ICSI

Dr. Sandro Barbacini, SBSItalia

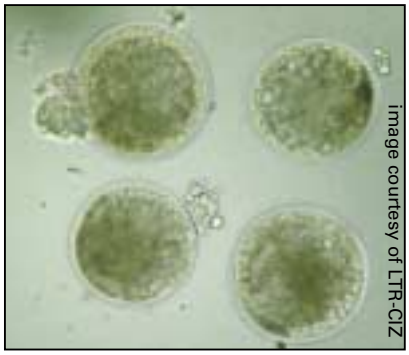
image courtesy of LTR-CIZ

9 days equine blastocysts produced by ICSI

For many years Artificial Insemination (AI) and Embryo Transfer (ET) have been the most widely used assisted reproductive techniques (ART) in the horse breeding industry, while other procedures based on in vitro production of equine embryos have emerged only in the last few years.
In fact, a few recent scientific reports showed that it is possible to obtain pregnancies and live foals after collection of immature oocytes by Ovum Pick Up (OPU) followed by in vitro culture (IVC) for their maturation and fertilization by Intracytoplasmatic Sperm Injection (ICSI) of a single sperm. These embryos can immediately be transferred to synchronized recipients or frozen for later transfer. OPU is a technique that allows oocytes to be collected

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SBS Affiliate Focus

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Mountain States Equine Reproduction Services

>> For more information:

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Farmington, Utah 84025

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Mountain States Reproduction Services, LLC (MSRS) in Farmington, Utah is situated between the Great Salt Lake and the Wasatch Mountains at the beautiful Buffalo Ranch equine complex. MSRS offers equine semen & embryo freezing services to stallion & mare owners in Utah, Idaho, Nevada, Wyoming, Colorado and other surrounding areas. The Facility's Export Certified Reproduction Laboratory allows MSRS all the needed technology and quality control procedures necessary to carry out its mandate. The reproduction laboratory consists of a dedicated AV prep room, large collection room with adjustable, beveled-end phantom, 4-stock palpation room, embryo flush room, embryo handling room, and a semen processing room that houses computer integrated sperm counter, CASA system for motility analysis, programmable cell freezer and semi-automatic straw filler and sealer. The compartmentalization and rooms dedicated to specific procedures affords MSRS the ability to run a fully certified export lab while still allowing for an efficient daily processing flow.

The combination of state-of-the-art instruments and a staff trained in cutting edge assisted reproduction procedures allows MSRS to offer the highest quality equine reproductive management available. MSRS offers a full battery of stallion and mare reproduction options. Mare services include reproductive examinations, ultrasonographic pregnancy detection and fetal sexing, insemination with fresh, cooled and frozen semen, embryo transfer utilizing their own receipent herd, oocyte retrieval and transfer, and superovulation using eFSH. Additional stallion services include reproductive examinations, semen collection, evaluation, shipping and freezing, and epididymal sperm recovery and freezing. Storage and distribution management of frozen semen is also available.

Emerald Ridge Farm

Ontario, Canada

Emerald Ridge Farm is located on 134 acres in Wellington County (just immediately outside of Guelph) in the "heart" of horse country.

Originally founded in 1998, the business has flourished to the point that it is a full service breeding facility offering a separate stallion barn with accommodation for resident stallions, complete with a laboratory and breeding shed. The main barn consists of 32 stalls which houses temporary and full time resident mares. During the breeding season, an additional farm is used to accommodate overflow.

Emerald Ridge Farm is owned and operated by Dr. Patrick Meyers and his wife, Anna DeMarchi-Meyers. Dr. Patrick Meyers obtained his veterinary degree from the Ontario Veterinary College in 1984, completed his residency in large animal theriogenology at Texas A & M University, becoming a board-certified theriogenologist (reproduction specialist) in 1989.

Anna DeMarchi-Meyers obtained a Bachelor of Science degree with a major in Animal Science from the University of Guelph in 1988. She has worked extensively in the veterinary pharmaceutical field leaving the industry in 2003 in order to focus her efforts on the business. She continues to free lance, writing horse health related articles for Horse Care Magazine and the Harness Edge.

In 2006, Emerald Ridge Farm partnered with Kentuckiana Farms, Georgetown, Kentucky and presently manage a roster of high quality standardbred stallions. Dr. Patrick and Anna Meyers are excited about becoming the first SBS affiliate in Canada and look forward to providing and expanding semen freezing services to clients.

>> For more information

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Cutting Edge Reproductive Technology

Ovum Pick Up and ICSI

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directly from ovarian follicles by use of a trans-vaginal ultrasound probe. This technique was first described about 15 years ago and has been subsequently refined by American and Australian researchers. OPU has been proven safe and repeatable in mares and can be performed for 5-6 consecutive cycles and every 10-15 days without causing particular collateral effects.

For the collection procedure the trans-vaginal ultrasound probe is introduced into the donor mare's vagina and the ovary is positioned against the transducer face by manipulations per rectum. A special needle is then advanced into the ultrasound probe and used to puncture and vigorously flush the ovarian follicles. The procedure is then performed on the opposite ovary.

Today the most practical use of OPU is to recover in vivo matured oocytes for oocyte transfer. This technique involves the recovery of the oocyte from a pre-ovulatory follicle and its transfer to an inseminated recipient mare at the time that it would have been ovulated if left in the donor mare. It has been shown by Carnevale and co-workers in Colorado that the use of this procedure results in satisfactory pregnancy rates except for intrinsically compromised oocytes collected from older mares.

Above:
First Commercial Foal born from and embryo produced by ICSI and transferred after freezing-thawing. Foal & Recipient Dam at 7 days of age born in 2005 – Sire Argentinus, Dam Zeus, both Warmblood. Recipient is a Haflinger Mare.

Below:
The same foal at 2 years.

ICSI is a modern laboratory technique that accomplishes in vitro fertilisation (IVF) by injecting a single spermatozoa into the oocyte.

Although normal numbers of fertile sperm are needed for oocyte transfer to be successful, ICSI provides a method to obtain offspring by using semen with low motility and/or poor reproductive performance in vivo. When used in combination, OPU, IVC and ICSI have the considerable advantage of not requiring any hormonal stimulation of the donor and this aspect is of particular importance in mares because superovulation still gives inconsistent results.

In 2001, Select Breeders Service Italia started a collaboration with Laboratorio di Tecnologie della Riproduzione - CIZ (LTR-CIZ), an internationally recognized laboratory where a group of scientists headed by Dr. Cesare Galli conducts research in the fields of biotechnology and ART of many domestic animal species. This collaboration was initiated with the goal of establishing an OPU-ICSI-IVC technique that could lead to consistent commercial results.

ICSI: injection of a spermatozoa into a matured equine oocyte

Following a series of preliminary experiments, LTR-CIZ and SBSItalia performed 57 commercial OPU sessions on 35 donor mares aged between 3 to 24 years during the 2004 - 2007 seasons. The majority of the mares (25) were Warmblood, while 5 were Quarter Horses, 1 Paint Horse, 2 Standardbred and 2 AngloArab. Frozen-thawed semen from 27 stallions of varying quality and fertility was used. During the breeding season mares were subjected to OPU in diestrus in the absence of a dominant follicle if at all possible. All the ovarian follicles ranging from 0,5 to 4 cm diameter were aspirated by OPU and the recovered oocytes were then matured in vitro. The matured oocytes were fertilized by ICSI and allowed to develop to the blastocyst stage in vitro. The blastocysts were frozen in media containing 10% glycerol and subsequently stored in liquid nitrogen. Embryos were frozen on day 6, 7, 8 or 9 after ICSI in relation to the time when the blastocyst stage was achieved. Embryos were transferred non-surgically to recipient mares 4 to 6 days (preferably 5 days) after spontaneous ovulation. During the 57 OPU sessions, 953 follicles were aspirated and 559 oocytes were recovered. Of these, 366 (66%) oocytes matured and were fertilized by ICSI giving rise to 49 blastocysts (0.85 blastocysts per OPU-ICSI-IVC session).

To date, 35 thawed embryos were non-surgically transferred and 19 recipient mares were

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It Only Takes One...

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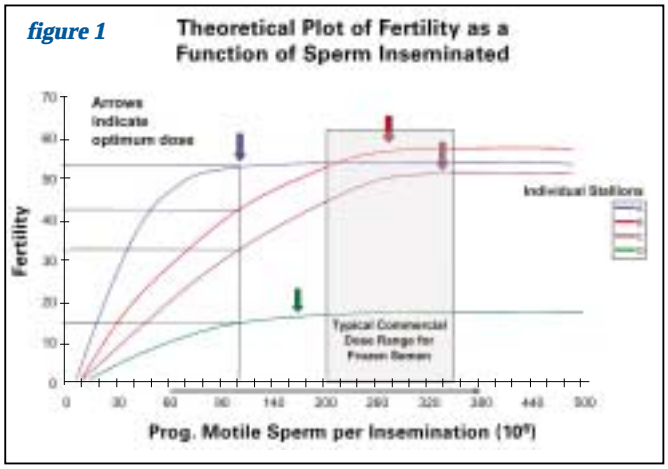
curve (see figure1). However, the slope of the curve and the maximum level of fertility are different for individual stallions.

In figure 1, stallion A achieves maximum fertility with much fewer sperm per insemination than the other 3 stallions. Insemination of more sperm for this stallion does not further increase fertility. The appropriate dose for this stallion would be 100 million progressively motile sperm. Stallion B has a higher maximum level of fertility but it is only achieved following insemination of far more sperm. When inseminating 100 million sperm for all 4 stallions in our example, a wide range of fertility is obtained (53% for A, 42% for B, 32% for C and 15% for D). Increasing the number of sperm inseminated to 250 million for stallion A does not change fertility while increasing to 250 million sperm for stallions B and C results in a significant increase in fertility. Stallion C is capable of achieving similar fertility as stallions A and B however reaching this level of fertility requires the insemination of far more sperm. Stallion D has a low level of maximum fertility that cannot be overcome by insemination of far greater numbers of sperm. This is due to the fact that some defects of sperm are compensable and others are non-compensable (see sidebar).

figure 1 **Theoretical Plot of Fertility as a Function of Sperm Inseminated**



Why do sperm from different stallions inseminated into mares under the same management conditions have such a wide range of fertility?

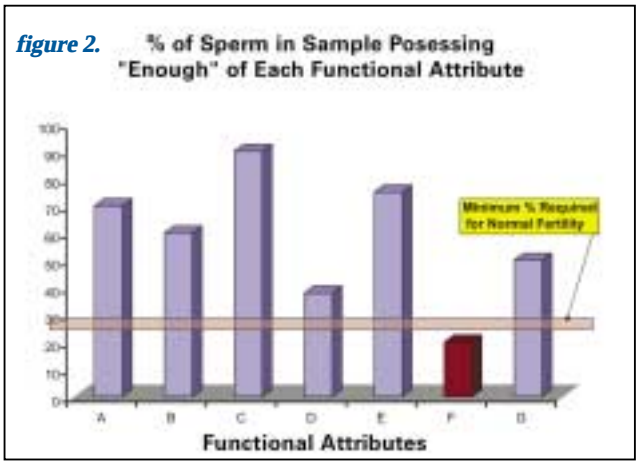
Fertility is a result not simply of the total number of sperm inseminated but rather the number of functionally competent sperm inseminated. A functionally competent sperm must possess "enough" of each of the many functional attributes required for fertilization. A simple example is sperm motility. Most would agree that a sperm must be motile in order to be fertile following standard uterine insemination. So a stallion with 30% motility would require insemination of twice as many sperm as a stallion with 60% motility in order to achieve the same fertility. This simple example would only be true IF sperm motility were the only functional attribute required for fertilization. Unfortunately this is clearly not the case. Sperm motility (regardless of how sophisticated it is measured) does not equal fertility. A sperm that is not motile is likely not fertile but a sperm that is motile may or may not be fertile. In addition to progressive motility, a fertile sperm must possess acceptable morphological characteristics,

Compensable vs. non-compensable defects.

"If 30% post-thaw motility is acceptable and this sample has 15%, then why can't you just double the number of straws inseminated?"

For some stallions you can... but for others you can double, triple or increase by 10-fold the number of sperm inseminated without increasing fertility. In figure 1 we saw that doubling the number of sperm from 100 million to 200 million for stallions B and C would significantly improve fertility but have no effect on fertility of stallion D. All semen samples contain some defective sperm. The ratio of defective to functional sperm in the sample determines the fertility of that sample. However not all defects are alike. Some defects impair the sperm's ability to penetrate the oocyte and initiate fertilization. These sperm never participate in the fertilization process and therefore do not compete with the other fully functional sperm in the sample. These defects are said to be "compensable" because one can compensate for low fertility of the sample by increasing the total number of sperm inseminated until the threshold number of fully functional sperm is reached. Other defects do not prevent the sperm from binding to and penetrating the oocyte rather; these defects affect the process after fertilization is initiated and lead to early embryonic death. These sperm compete with fully functional sperm to be the one sperm that fertilizes the oocyte. Increasing the total number of sperm inseminated does not increase the chances of fertilization by a fully functional sperm because the ratio of defective to functional sperm is still the same. Therefore in this case lower fertility can not be compensated for by increasing the total number of sperm inseminated.

intact plasma and acrosomal membranes in order to bind to and penetrate the oocyte and a whole host of other known and unknown functional attributes. See figure 2 for an illustration of this concept.



Most laboratories package semen doses based on the number of progressively motile sperm and in some cases take into account the percentage of sperm with "normal" morphology. Including adequate numbers of sperm that are normal in these two functional attributes still does not guarantee fertility (figure 2.) Another challenge to standardizing semen quality is the ability to accurately and precisely measure semen quality with standard laboratory assays. Frozen semen is often sold with a guarantee (or non-guaranteed claim) that after thawing each dose will contain a minimum of "*n*" million total sperm with "*x*"% progressive motility Measuring sperm motility in many laboratories is performed using a subjective estimation of the percentage of sperm in a sample that are moving in a "progressive fashion". Such subjective estimates are prone to technician bias and are typically less precise and less accurate than computer assisted sperm analysis (CASA) methods of measuring motility. Accurately measuring sperm concentration in both the initial sample and after centrifugation and dilution in extender is also critical if the resulting dose is to contain the correct number of sperm. Inaccuracies due to improper techniques and instrumentation used for counting sperm can lead to improper sperm concentration in the doses packaged for commercial distribution. For more information on how SBS ensures quality read Quality Control is at the Core of the SBS Difference from the Fall 2006 issue of Foundations.

Ideally, commercial producers would determine the number of sperm required for each stallion to achieve maximum fertility and prepare doses of semen accordingly. This is impossible in horses as far too many mares would need to be inseminated under controlled conditions with doses of varying amounts to establish the number of sperm per dose required for maximum fertility. As a result, most doses contain more sperm than are required. In figure 1 if sperm from each of these stallions was packaged at 300 million motile sperm per dose, maximum fertility for all would be achieved.

What other factors influence the probability that a given mare will become pregnant following insemination with semen from a given stallion?

To this point we have focused mainly on the male factors that contribute to fertility however the probability that a mare will become pregnant following insemination is dependant upon numerous other variables. Remember, fertility is the product of (stallion fertility [or fertility of that semen sample]) times (mare fertility [or fertility of the

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Figure 2

Let's assume that there are 7 functional attributes of a sperm that are required for successful fertilization (attributes A-G; A= motility, B = morphology, etc) and that we have laboratory assays that are capable of accurately measuring these attributes and distinguishing between normal and abnormal. (This is clearly a gross oversimplification as there are likely many more attributes required and our ability to accurately measure them and relate them to fertility is lacking.) If a sample contains mostly sperm that are normal in attributes A, B, C, D, E, and F and abnormal in G, the sample will be infertile. Likewise if a sample has mostly sperm normal in A, B, C, D, E and G and abnormal in F, the sample will be infertile. Measuring only motility is measuring only A. Measuring motility and morphology is measuring only A and B and so on. In this example, most laboratories would reject this subfertile sample only if attribute F was motility or morphology. Predicting that a semen sample will be fertile requires the ability to measure all of the functional attributes. The only realistic goal of semen evaluation then is to try and predict that a given semen sample or semen from a given stallion is likely to be subfertile because it is "abnormal" in one of the attributes that we can measure.

For more details on this topic
www.selectbreeders.com

It Only Takes One...

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oocyte ovulated during that particular cycle) times (all other variables). See the accompanying sidebar for an illustration of this concept.

Many claim that the only true test of fertility of frozen semen (this actually applies to all semen, fresh or frozen) is to inseminate mares and measure pregnancy outcome. Actually, the only thing that can practically be determined with horses is whether or not semen from a particular stallion is capable of achieving a pregnancy. Some breeders will report pregnancy rates following insemination of 5 or 10 or 20 mares as demonstration that a particular stallion is either highly fertile or subfertile. Since observed fertility after insemination is a binomial function (either she is pregnant or not) it is like flipping a coin. If you flip a coin and measure the results you will find that the more times you flip the coin (observations) the closer you will get to the true probability (50%) of heads or tails in your measured result. For example, two stallions (A and B) of equal fertility (50%) are used to inseminate 10 mares each. The observed fertility of stallion (A) is 70% and the observed fertility of stallion B is 30%. Stallion A is statistically no more fertile than stallion B. This imprecision in the observed measurement of true fertility is due to binomial variation alone. In other words, there is an equal chance that if those same stallions were to breed another 10 mares each, stallion A would get 3 of 10 pregnant and stallion B would get 7 of 10 pregnant. So, as the number of matings (observations) is increased the observed result is closer to the true fertility.

For more details on this topic
www.selectbreeders.com

So what does all this mean?

The take home messages here are:

1. Fertility is a complex process that is dependant upon numerous factors associated with the stallion and mare. Many of these factors are known but difficult to accurately measure and many other factors are likely important but unknown.

2. The optimum number of sperm per insemination is different for individual stallions and producers should strive to package sperm in doses well beyond the critical number required for maximum fertility to account for the effect of uncontrolled variables on fertility in the field.

3. Standard laboratory evaluations of semen quality can be misleading and may be subject to bias and inaccuracies.

4. The fertility of a semen sample cannot be predicted based on the results of standard laboratory assays. All laboratory assays are measuring some aspect of relative cell health but do not predict fertility.

5. The goal of evaluating semen quality should be to accurately and precisely measure attributes to identify stallions or semen samples that are likely to result in very poor fertility. Those samples or stallions can then be culled from the commercial breeding population.

6. Mare owners entering into an agreement to breed a mare to a particular stallion via cooled or frozen semen should be aware of the numerous factors that could influence the outcome. Purchasing frozen semen by the dose and without any guarantee of fertility or minimum semen quality is risky business and truly "buyer beware". If semen is purchased "by the dose" buyers should insist on a guarantee of a minimum number of sperm per dose and a minimum percentage of progressive motility after thawing. SBS recommends that frozen semen be used as a tool for efficiently delivering semen to a mare in fulfillment of a contract for pregnancy between a mare owner and stallion owner.

The Mathematics of Fertility

For the purpose of this example, let's ignore the very significant effect of "other variables" and assume:

Observed Fertility = (stallion fertility) X (mare fertility)

So, an observed fertility of 50% (0.50) could be the result of stallion fertility of 55% times mare fertility of 90% or stallion fertility of 90% times mare fertility of 55%. Even when a highly fertile stallion is bred to a highly fertile mare (each with 90% fertility) the probability of that mating resulting in a pregnancy is not 100% (0.9 X 0.9 = 0.81 or 81%). And remember we are ignoring the significant "other variables" that influence the outcome. Breeding an older, perhaps subfertile mare to a subfertile stallion (or semen with poor quality) really decreases the probability of obtaining a pregnancy efficiently. Mare fertility (40%) X stallion fertility (40%) = 16% probability of pregnancy.

Ovum Pick Up and ICSI Continued

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detected pregnant by ultrasonography. Three pregnancies resulted in early embryonic losses before 30 days of gestation and two more were lost before 60 days of gestation. Ten foals have been born and 5 pregnancies are ongoing. One foal was lost at birth and one died 10 days after birth following a neonatal septicemia.

The results obtained in this short period of commercial application suggest that the use of OPU-ICSI-IVC can lead to reasonable results and may have applications for highly valuable mares and stallions. Nevertheless, OPU-ICSI-IVC likely will not overcome the use of conventional Embryo Transfer because of the specialized equipment and skilled personnel needed to perform such procedures.

Advanced reproductive techniques such as OPU,ICSI, and IVC add to the tools available to breeders when it is necessary to:

- Obtain pregnancies from repeat breeder mares
- Obtain pregnancies from mares with reproductive problems like pyometra, degenerative endometriosis or irreparable cervical lacerations
- Obtain pregnancies from mares that are not able to carry a foal to term
- Obtain pregnancies by producing and freezing embryos for later transfer during the spring or fall transitional phase when large number of follicles can be detect ed on the mare's ovaries.
- Use frozen-thawed semen of very poor post-thaw quality or very limited in vivo fertility.

Another SBS Laboratory Adds Mobile Services

Sweden's New Mobile Semen Freezing Laboratory

Select Breeders Service Sweden announces their new mobile equine semen freezing laboratory. With this new laboratory Dr. Kerstin Darenius can now offer semen freezing to stallion owners at their home facilities. SBS Sweden's mobile service provides a convenient alternative for stallion owners wanting to keep their stallions at their home stud for training. This mobile laboratory is European Union approved for semen freezing.

This fully equipped laboratory is available now. For more information and to schedule the SBS Sweden mobile freezing laboratory contact Dr. Darenius at 011-614 11 or kerstin_darenius@yahoo.se

Frozen Semen At Work: Topgun Whiz

1995 QH Topsail Whiz - Ms Tidy Jac

Topgun Whiz will be exported to Italy for the 2008 breeding season. Owners Arcese Quarter Horses, Canciani and Bruni have stood the stallion in the US for the past year where the stallion has bred a large book of mares with both cooled and frozen semen. Semen frozen by Select Breeders Southwest (Aubrey, TX) was also exported to Italy where it was distributed throughout Europe resulting in many successful breedings. Italian based owner Arcese was also able to utilize the frozen semen on its Northern Italian based herd. With the stallion now standing in Italy a bank of frozen semen is being maintained at SBSW for distribution to US mares in 2008. Through an efficient frozen semen breeding program Arcese Quarter Horses and the stallion's other owners are able to benefit from Topgun Whiz's popularity in both Europe and US markets.

Promote the Quality Behind Your Breeding Program

The SBS logo is available for use in your print advertisements and website. Email info@selectbreeders.com with your stallion's name and we will send one along for you to use.

Harmony's Rousseau

Dutch National Champion and PAVO-Cup winner Harmony's Rousseau services mares in the US and EU via efficient and cost effective frozen semen. Licensed by Hanoverian, Oldenburg, and Westphalian Verbands, Rousseau's influence has spread into other European countries via cooled and frozen semen making him the leading producer of riding horses in the Netherlands. While in Holland and Germany, Rousseau bred over 1000 mares. During those years semen was frozen by Select Breeders Affiliates from Italy and Northern Germany and imported to North America by SBS-Maryland for insemination of mares in the U.S. Harmony Sporthorses had been partners in the stallion since 2003 and with their full acquisition, Rousseau was imported to the U.S. in 2006. In 2007 Harmony's Rousseau bred mares in the US via cooled and frozen semen for the first time, while he was still being offered in Holland and Germany via exported semen frozen by SBS - Maryland.

SBS Clients effectively utilize frozen semen to expand their stallion's markets.

The SBS Breeder's Support Program is designed to assist stallion owners in expanding to unfamilar markets. Ask your local SBS Affiliate for more information.

Search for Stallions at www.siredirectory.com

Are you breeding AQHA, APHA or ApHC?

Keep up to date on rules regarding the use of frozen semen in your breed organization.

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