

# Practical Use of Frozen Semen in an Equine Breeding Program

**Specific Application to the Australian Standardbred  
Breeding Industry**

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**An International Specialised Skills Fellowship**

Sponsored by Agrifood Skills Australia





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# i. EXECUTIVE SUMMARY

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The introduction of Artificial Insemination revolutionized the Standardbred Breeding Industry. Embracement of the use of artificial breeding techniques produced a shift in the breeding landscape from the previous system of natural live cover to today's position of more than 98 per cent of foals being conceived through artificial insemination. Central to this transition was the education of owners and breeders regarding the advantages and best practice models for the use of these techniques.

Currently frozen semen services constitute a small percentage of the total number of mares served. Importantly however, given the significant interest and expansion of trotters in the Australian racing scene, frozen semen is seen as is the growth area in the breeding sector, allowing for improved genetic diversity with access to leading European and American gene pools - in particular those elite world class performers that command such a fee as to make physical movement unfeasible. Foreseeably the use of frozen semen will continue to increase as it becomes accepted as a viable alternative to the physical movement of stallions in the face of rising importation and quarantine fees and risk of injury or disease. And we have now seen that a Stallion can successfully service a commercial book of mares with frozen semen. In season 2013/2014, the Stallion 'Somebeachsomewhere' (USA) served 116 Australian and 88 New Zealand mares with frozen semen for a total book of 204 mares in Australasia.

Jane Hearn was privileged to be awarded the 2014 ISS Institute Agrifoods Skills International Fellowship. The purpose of this fellowship was to perform a practical consideration of the current International practices and methodologies in the use of frozen semen in an equine breeding program, specifically applicable to the Standardbred Breeding Industry. The Fellow travelled to Sweden in June 2015 to meet with highly regarded Equine Reproduction Specialists, experienced in the use of frozen semen. The various destinations visited were selected to provide a cross-section of the Standardbred breeding environment in that country.



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## ii. ABBREVIATIONS/ACRONYMS

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<b>AI</b>	Artificial Insemination
<b>EU</b>	European Union
<b>GnRH</b>	Gonadotropin Releasing Hormone
<b>GVEH</b>	Goulburn Valley Equine Hospital
<b>HBV</b>	Harness Breeders Victoria
<b>hCG</b>	Human Chorionic Gonadotropin
<b>HRA</b>	Harness Racing Australia
<b>HRV</b>	Harness Racing Victoria
<b>MR</b>	Mile Rate
<b>NaCl</b>	Sodium Chloride Solution (Saline)
<b>SBS</b>	Select Breeders Services



## iii. DEFINITIONS

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**Artificial Insemination:**

The procedure of delivering collected semen directly into the uterus via a pipette introduced through the vagina and passed through the cervix. The semen sample may be fresh, chilled or frozen.

**Broodmare:**

Female horse (mare) retained solely for the purpose of breeding.

**Broodmare Base:**

A Stud Farm dedicated solely to the management of the mare, no stallions stand on the farm.

**Collection:**

The procedure of obtaining an ejaculate from the stallion.

**Conception Rate:**

The percentage of mares served that return a positive pregnancy result, can be further defined as

1. Per Cycle Conception Rate: the percentage of mare breeding cycles that result in a positive pregnancy result. A mare may be served multiple times within a cycle.
2. First Cycle Conception Rate: the percentage of mares in foal (returning a positive pregnancy test) after having been served on the first cycle.
3. Seasonal Conception Rate: the percentage of mares that are in foal at the end of the breeding season.

**Extender:**

A fluid volume that is added to the semen sample to nourish the sperm cells or protect them from damage. Extenders contain antibiotics to prevent or control bacterial growth in the sample, and/or cryoprotectants to protect the sperm cells from cellular damage.

**Induction (of Ovulation):**

Ovulation may be stimulated by administration of an induction agent (hormone). For ovulation to be induced and to be predictable, the agent must be administered at the appropriate stage of oestrus. The ovulation induction agents currently available for use in Australia are the GnRH analogues Deslorelin and Ovuplant, and hCG (Chorulon).

**Maiden Mare:**

A mare that has not previously been mated/inseminated.

**Morphology:**

Physical characteristics.

**Multi-parous:**

Having had multiple pregnancies.

### iii. DEFINITIONS

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#### **Pregnancy Rate:**

The percentage of served mares that record a positive pregnancy test (are pregnant).

#### **Pregnancy Testing:**

Pregnancy in the mare is routinely determined by ultrasound examination performed at:

1. 14-16 days (early test). The embryonic vesicle is easily discernible at 14 days.
2. 28 days (mid test). The embryonic heartbeat is visible and the embryo is examined for normal gross development and orientation.
3. 45 days (final test). The developing foetus is examined for normal development and the presence of a heartbeat.

#### **Service Fee:**

The fee charged by the Stallion Manager or owner for the stallion's contribution to achieving a confirmed pregnancy. The service fee is usually generated either upon a confirmed 45 day pregnancy or after the delivery of a live foal.

#### **Semen Concentration:**

The number of sperm cells in a semen sample, usually expressed as millions (of sperm) per millilitre.

#### **Transported Semen:**

A semen sample that is shipped to a location that is a distance away from that of the stallion for the purpose of inseminating a mare. Transported semen may be either:

**Chilled Semen** – the collected semen sample is cooled down and maintained at refrigerator temperature of five degrees centigrade. Cooling slows down the semen activity so that it uses little energy. The average lifespan of chilled semen is 72 hours.

**Frozen Semen** – the collected and processed semen is packed into straws (today most commonly ½ mL straws) and frozen in liquid nitrogen. Provided the straws are maintained correctly in a liquid nitrogen storage tank, the lifespan of the semen is indefinite.

#### **Twins (Twin Crushing):**

The presence of twin pregnancies in the mare is highly undesirable as the most common result is abortion (due to placental insufficiency) or birth of stillborn, malformed or malnourished foals. Multiple pregnancies are reduced to a single pregnancy by squeezing (twin crushing) or bouncing one of the embryonic vesicles to disrupt or rupture the vesicle membrane. The earlier this procedure is performed, the higher the rate of survival of the remaining pregnancy

# 1. ACKNOWLEDGEMENTS

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Jane Hearn would like to thank the following individuals and organisations who generously gave their time and their expertise to assist, advise and guide her throughout the Fellowship program.

## **Awarding Body – International Specialised Skills Institute (ISS Institute)**

The International Specialised Skills Institute (ISS Institute) is an independent, national organisation. In 2015 it is celebrating twenty-five (25) years working with Australian governments, industry education institutions and individuals to enable them to gain enhanced skills, knowledge and experience in traditional trades, professions and leading edge technologies.

At the heart of the ISS Institute are our individual Fellows. Under the Overseas Applied Research Fellowship Program the Fellows travel overseas. Upon their return, they are required to pass on what they have learnt by:

- Preparing a detailed report for distribution to government departments, industry and educational institutions
- Recommending improvements to accredited educational courses
- Delivering training activities including workshops, conferences and forums.

Over 300 Australians have received Fellowships, across many industry sectors. In addition, recognised experts from overseas conduct training activities and events. To date, 25 leaders in their field have shared their expertise in Australia.

According to Skills Australia's 'Australian Workforce Futures: A National Workforce Development Strategy 2010'.

*Australia requires a highly skilled population to maintain and improve our economic position in the face of increasing global competition, and to have the skills to adapt to the introduction of new technology and rapid change. International and Australian research indicates we need a deeper level of skills than currently exists in the Australian labour market to lift productivity. We need a workforce in which more people have skills and knowledge, but also multiple and higher level skills and qualifications. Deepening skills and knowledge across all occupations is crucial to achieving long-term productivity growth. It also reflects the recent trend for jobs to become more complex and the consequent increased demand for higher-level skills. This trend is projected to continue regardless of whether we experience strong or weak economic growth in the future. Future environmental challenges will also create demand for more sustainability related skills and knowledge across a range of industries and occupations.*

In this context, the ISS Institute works with our Fellows, industry and government to identify specific skills and knowledge in Australia that require enhancing, where accredited courses are not available through Australian higher education institutions or other Registered Training Organisations. The Fellows' overseas experience sees them broadening and deepening their own professional knowledge, which they then share with their peers, industry and government upon their return. This is the focus of the ISS Institute's work.

For further information on our Fellows and our work see <http://www.issinstitute.org.au>.

The Fellow also warmly thanks the CEO (Lou Ellum) and staff (Ken Greenhill, Paul Sumner, Danielle Cull and Fiona Waugh) of ISS Institute for their assistance in the planning and development of the Fellowship and completion of this report.

## 1. ACKNOWLEDGEMENTS

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Agrifoods Skills Australia was the Industry Skills Council for the Agifoods Industry: the rural and related Industries, food processing (including beverages, wine and pharmaceuticals), meat, seafood, and racing. The Fellow would like to thank them for providing funding support for this Fellowship.

### **Mentors and Supporters:**

- Andrew Kelly, Chief Executive Officer, HRA
- Isabella Galati, Human Resources Manager, HRV
- Rod Pollock
- Tony Britt, President, HBV
- Dr Angus McKinnon, Goulburn Valley Equine Hospital
- Donna Egan & David James, Empire Stallions
- Duncan McPherson, Aldebaran Park

### **Employer Support:**

The Fellow would like to acknowledge the support of her previous employer, Mrs Helen Head of Grenada Park Standardbreds. In addition, the Fellow would also like to acknowledge her business and life partner John Nalder of Dunroamin Standardbreds.

## 2. ABOUT THE FELLOW

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From a young age Jane Hearn has been passionate about horses. Growing up near Corryong in Victoria's High Country she was an active member of the Upper Murray Pony Club, taking every opportunity to participate in a wide variety of equestrian pursuits. Hearn graduated from Corryong Secondary College, before studying at the University of Melbourne, completing her Bachelor of Science (Zoology).

After completing her degree Hearn spent several years developing her skills with horses. Relocating to the Goulburn Valley, she secured a position with Jim Vasey and Angus McKinnon of the Goulburn Valley Equine Hospital (GVEH). A large equine specialist referral clinic and teaching hospital for both Australian and International veterinary students and graduates, GVEH provided her invaluable and career defining experiences. A leading Equine Reproduction Specialist, Dr McKinnon was then developing new techniques in equine reproduction such as follicular aspiration (the harvesting of eggs directly from the ovary of a live donor mare), in-vitro fertilization, sperm injection, and embryo transfer.

Wanting to pursue a career in Equine Breeding, Hearn took up a position with Alabar Bloodstock at Echuca, Victoria. Alabar Farm is the largest Standardbred Stud in Australasia, at that time accommodating approximately 350 resident mares and building to 900 mares on farm at the height of the season. The stud stood 13 Australian based stallions and an additional international roster of stallions based at sister farms in New Zealand (Alabar NZ) and Canada (Tara Hills). Over several seasons she continued to develop her skills as a broodmare manager. Additionally, she managed the transported semen program for off-farm mares –booking mares for service, receiving and collating orders for the day, assisting with laboratory preparation of and packing the semen for transport, notifying studs and owners of flight details, developing transport pathways and tracking pregnancy results. While in its infancy then, transported chilled semen today accounts for the majority of all services in the Standardbred Breeding Industry.

When offered the opportunity to manage 'Wildwood Farm' with her partner, John Nalder, the couple relocated to the Hunter Valley. Managed by Alabar as part of a negotiated package for the frozen semen breeding rights to several leading European trotting stallions, Wildwood was owned by prominent French Harness Driver and Trainer Jean Pierre Dubois. In addition to Dubois' six freshly imported thoroughbred mares booked for service at several local Studs (Coolmore, Widden and Arrowfield), the farm was operated as a commercial Standardbred Stud. At the end of the season the couple returned to Echuca and Hearn completed the Certificate of Equine Artificial Insemination through Glenormiston College (University of Melbourne).

In 2001 the couple's son Sam was born, followed by two daughters, Georgia and Caitlyn, in 2003 and 2004 respectively. Hearn then took up the position of Studmaster for Mrs Helen Head of Grenada Park Standardbreds at Manna Lodge at Congupna, Victoria. In 2016 Hearn and Nalder commenced trading full time as Dunroamin Standardbreds, maintaining a dedicated broodmare base (stud farm) where they offer equine breeding services –supervised foaling, breeding via Artificial Insemination with chilled and frozen semen, weaning, weanling education and preparation of yearlings for the commercial Yearling Sales market. Additionally the couple breed, educate and train Standardbred horses for sales and racing. Hearn is responsible for designing and implementing the stud breeding programs and through sound handling and management practices and attention to detail is able to maintain high per cycle and seasonal conception rates.



## 3. AIM OF THE FELLOWSHIP PROGRAM

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The focus of all ISS Institute Fellowships is on applied research and investigation overseas by Australians. The main objective is to enable enhancement and improvement in the skills, knowledge and practice not currently available or implemented in Australia and the subsequent dissemination and sharing of those skills and recommendations throughout the relevant Australian Industries, Educational Institutions, Government bodies and the community.

The aim of this Fellowship was to perform a practical consideration of the use of frozen semen applicable (but not restricted) to the Standardbred Breeding Industry, with the objective of improving reproductive efficiency and productivity and minimizing reproductive waste.

The areas of applied research for this Fellowship are therefore defined as the following elements.

1. Review current best practice methods for the practical use of Frozen Semen, including:
  - » Preparation of the mare for service
  - » Artificial Insemination techniques and protocols
  - » Semen thawing techniques
  - » Post-service mare management.
2. Identify key requirements for optimal conception rates, considering:
  - » The broodmare nominated for service with frozen semen
  - » The stallion entering a collection and freezing program



## 4. THE AUSTRALIAN CONTEXT

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The use of Artificial Insemination (AI) in the Standardbred Breeding Industry dramatically changed the breeding landscape. By utilizing transported chilled and frozen semen, mare owners were suddenly afforded the option of breeding their mares close to or at home and to a stallion of their choice, irrespective of the sire's geographical location. AI offered the mare owner enormous savings in transport and agistment costs and negated the necessity of exposure of valuable breeding stock – heavily expectant mares or young foals – to the risk of injury during transport or physical service. Stallion owners no longer required large holdings to accommodate incoming mares and could service multiple mares from a single ejaculate reducing the physical burden on the collecting stallion. The use of AI also offered improved biosecurity conditions in a reduction of the transmission of infectious venereal and non-venereal disease through limitation of horse movement and/or contact.

Additionally, the use of frozen semen affords breeders and Studmasters access to leading world-class performers and bloodlines, allowing for further expansion of the gene pool. At a pivotal stage in the growth of Australian Harness Racing, where we are seeing emerging international exchange of the Harness Racing Product through televised racing and wagering with the major European market, the use of frozen semen enables the production of an Australian bred product that is recognisable and competitive in an international (global) market. With increasing financial pressure in the form of rising quarantine and transportation costs, as well as the associated risk of injury, sickness or disease, the idea of servicing an overseas mare population without requiring physical movement of the stallion can be very attractive to those stallion owners contemplating dual-hemisphere stud duties for their stallions.

The biology of equine reproduction is the same across all breeds of horses – so then is the transfer of knowledge. This project, whilst being undertaken in the interest of the Standardbred Breeding Industry, is relevant to all equine codes that accept the use of artificial breeding techniques. Conversely, methodology and procedures employed by other equine codes may be drawn upon to strengthen the position of Harness Breeding.



## 5. IDENTIFYING THE SKILLS AND KNOWLEDGE ENHANCEMENTS REQUIRED

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In 2011, in recognising the extreme pressure facing breeders, the HRA Executive established the Australian Standardbred Breeding Panel to determine the current health and trends of the Australian Standardbred Breeding Industry and to develop a plan to stimulate future growth of breeding and ownership of broodmares and racehorses. In part The Panel found that:

- The active broodmare population is 12,191, of which 7,800 are bred each year.
- 73% of breeders are hobbyists, while a further 20% are part-time breeders. As result many breeders are time poor or inexperienced, contributing to breeding inefficiencies, and are particularly susceptible to rising costs.
- Rising cost is the largest cause of breeders exiting the Breeding Industry or reducing their breeding activities.
- Inefficient breeding management and care is a major cost to the industry.
- Education, training and accreditation to improve performance across the various supply chain service providers would contribute to improved productivity and reduction in costs to The (Breeding) Industry.<sup>1</sup>

An Australian review of breeding records collected over multiple seasons (1990-2001) comparing the reproductive efficiency of Standardbred and Thoroughbred mare populations found the pregnancy rate per cycle to be significantly higher in Thoroughbred mares (70%) than in Standardbred mares (62%), while seasonal pregnancy rates were similar (a similar result in number of mares pregnant at the end of the season, but the Standardbred mares were served more times on order to obtain a pregnancy). The report suggested that while pregnancy rates were higher than previously reported, indicating improvement in reproductive management, the lower per cycle rate observed in the Standardbred population could be attributed to the fact that the majority of Standardbred Stud Farms were less likely to involve Veterinarians in routine breeding farm management.<sup>2</sup>

In order to attain repeatable success with frozen semen three areas need be addressed:

1. Selection of a suitable mare candidate.

Not all mares are likely to achieve a pregnancy with a frozen semen breeding program. Mares with post-service fluid clearance issues due to poor reproductive conformation, uterine scarring or poor pelvic-floor muscle tone (often associated with the aging or multi-parous mare), or those with a history of uterine inflammation or infection are generally considered to be unsuitable for service with frozen semen.

2. Quality of the frozen semen product.

Stallion fertility has a significant effect on conception rates. Frozen semen can be highly variable in quality, both amongst individual stallions and between collects.<sup>3</sup> Additionally, not all stallions will produce sperm that will survive the freezing/thawing process.<sup>4</sup> The quality of the frozen semen product is highly reliant on the control protocols instituted during the collection and production process. A thawed frozen semen sample should be of consistent sperm concentration, show normal sperm cell morphology and be free of bacterial contamination.

3. Experience and skill of the service provider.

The use of frozen semen is a specialised area of equine reproduction and requires specific

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<sup>1</sup> Australian Standardbred Breeding Panel, *Report on the Australian Breeding Industry*, July 2011

<sup>2</sup> Nath, L.C., Anderson, G.A., & McKinnon, A.O., (2010), 'Reproductive Efficiency of Thoroughbred and Standardbred horses in north-east Victoria', *Aust.Vet.J.* 88(5):169-75, May 2010

<sup>3</sup> Sieme, H., Harrison, R.H., & Petrunkina, A.M., (2008), 'Cryobiological determinants of frozen semen quality, with special reference to a stallion', *Anim.Reprod.Sci.*, 107(3-4):276-92.

<sup>4</sup> 4 Graham, J.K., (1996), 'Cryopreservation of stallion spermatozoa', *Vet. Clin. North Am. Equine Pract.* 12(1):131-47, April 1996.

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equipment for its storage and handling. Because of the limited lifespan of thawed frozen semen in the reproductive tract of the mare, the window for insemination in relation to mare ovulation is greatly reduced.<sup>5</sup> To achieve acceptable conception rates the mare need be inseminated within the period 12 hours prior to or within 6 hours post ovulation.<sup>6</sup> This necessitates an intensive approach to management of the mare, and in most instances involves induction of ovulation by hormone administration at the appropriate stage in the mare's oestrus cycle. To achieve acceptable conception rates with frozen semen then requires that the service provider be dedicated and sufficient proficiency in the ability to predict and manipulate the reproductive cycle.

### SWOT analysis:

#### Strengths:

- Within Australia we have highly skilled and qualified Veterinary and Academic resources, well credentialed in the use of frozen semen.
- Current literature suggests that insemination with frozen semen can achieve conception rates equal to those with chilled semen.<sup>7</sup>
- Racetrack success of frozen semen progeny continues to bolster breeder confidence in the product.
- The breeding sector of the Racing industry has great potential for economic impact for the state of Victoria, as breeders often sell their stock to interstate and overseas buyers. A strong breeding industry brings significant investment to Victoria from interstate and overseas buyers and owners. The harness breeding sector is responsible for generating more than \$54.0 million in the production and care of racing stock.<sup>8</sup> While a specific study of the Victorian Industry, these comments may be applicable to the national situation.

#### Weaknesses:

- There is an identified gap between the expert service provider and the novice or inexperienced breeder in the available training, skills development and accreditation.
- The cost to the mare owner of breeding with frozen semen is often prohibitive. As it is a specialized technique that requires specific equipment and knowledge and is labour intensive, not all Stud or Veterinary clinics will offer this service.
- A study completed by the fellow of estimated conception rates for frozen semen services in Australia found that:
  - a. Amongst stallions that served mares with both chilled and frozen semen in separate seasons, the conception rate for frozen semen services was lower than that for chilled semen services suggesting a need for a level of improvement in reproductive productivity (Table 1).
  - b. The estimated average seasonal conception rate for frozen semen services in the Australian Standardbred mare is consistently lower across all seasons than the conception rate for frozen semen services of 82% recorded in a recently published retrospective study of Northern

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<sup>5</sup> Barbacini, S., Zavaglia, G., Gulden, P., Marchi, V., & D. Necchi, D., (2000), 'Retrospective study on the efficacy of hCG in an equine artificial insemination program using frozen semen', *Equine Vet. Educ.*, 12(6):312-17.

<sup>6</sup> 6 Avanzi, B.R., Ramos Rdos, S., Araujo, G.H., Fioratti, E.G., Trinca, L.A., Dell'Aqua, J.A. Jr., Melo E Oña, C.M., Zahn, F.S., Martin, I., Alvarenga, M.A., & Papa, F.O., (2015), 'Fixed-time insemination with frozen semen in mares: is it suitable for poorly fertile stallions?', *Theriogenology*, 83(9): 1389-93, June 2015.

<sup>7</sup> Crowe, C.A.M., Ravenhill, P.J., Hepburn, R.J., & Shepherd, C.H., (2008), 'A retrospective study of artificial insemination of 251 mares using chilled and fixed-time frozen-thawed semen', *Equine Vet. J.*, 40 (6): 572-76, September 2008.

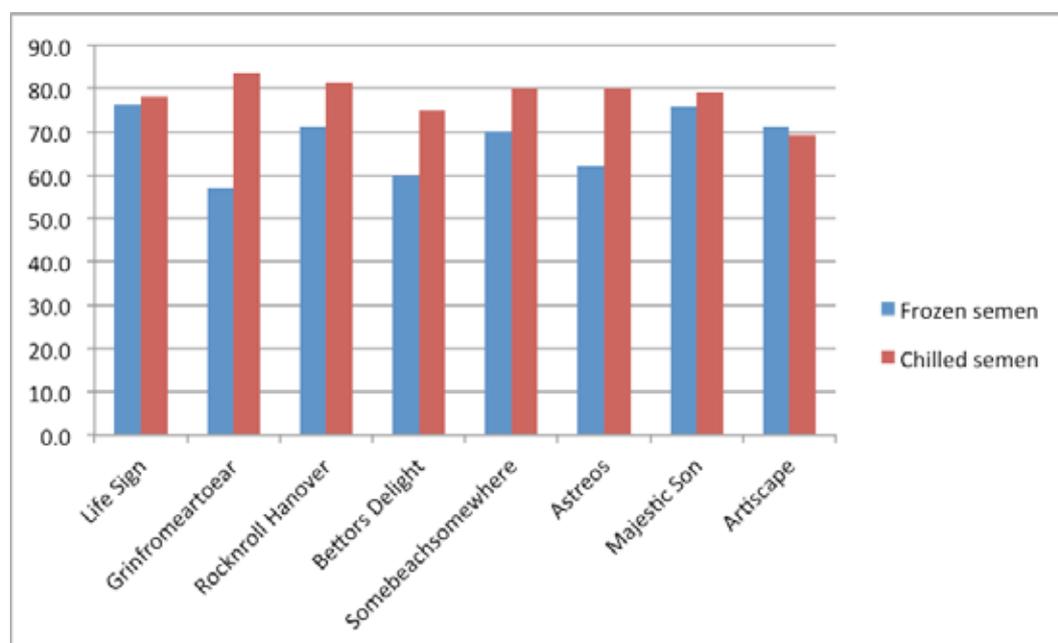
<sup>8</sup> IER Pty Ltd., (2013), *Size and Scope of the Victorian Racing Industry*.

## 5. IDENTIFYING THE SKILLS AND KNOWLEDGE ENHANCEMENTS REQUIRED

hemisphere mares (Table 2).<sup>9</sup>

- c. Conception rates vary significantly from stallion to stallion (Table 3). Variation in stallion fertility has long been recognised as a major factor in achieving a successful outcome with frozen semen.

Table 1: A comparison of estimated conception rates (%) for 8 stallions available in Australia in separate seasons with chilled and frozen semen.\*



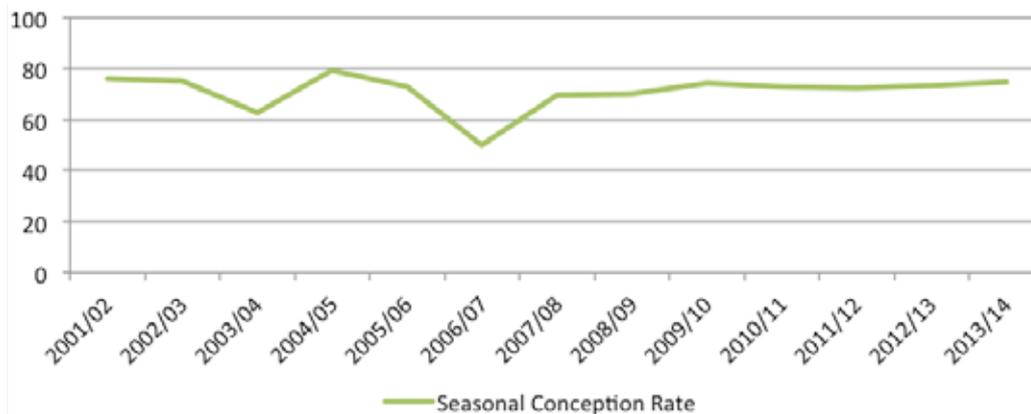
\*Data compiled from HRV database, using the Registered International Semen Transport Stallions Lists for multiple seasons, 1996/97 to 2013/14. The criteria for selection of the profiled stallion was:

- the stallion had served more than 20 mares in total in Australia with frozen semen
- the stallion had served mares in Australia with both frozen and chilled semen (in separate seasons).

<sup>9</sup> C.A.M., Ravenhill, P.J., Hepburn, R.J., & Shepherd, C.H., (2008), 'A retrospective study of artificial insemination of 251 mares using chilled and fixed-time frozen-thawed semen', *Equine Vet.J.*, 40 (6): 572-76, September 2008.

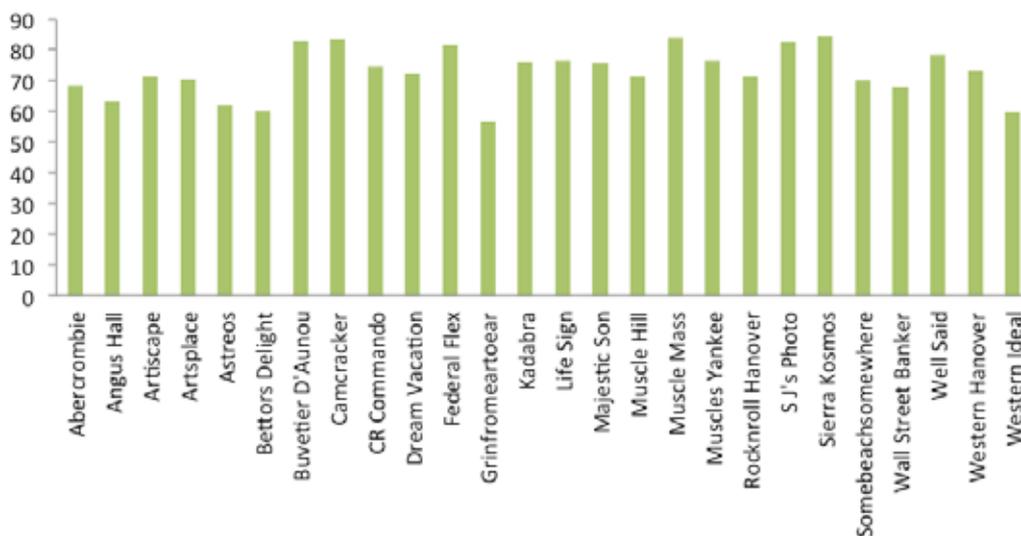
## 5. IDENTIFYING THE SKILLS AND KNOWLEDGE ENHANCEMENTS REQUIRED

Table 2: Estimated Frozen Semen Seasonal Conception Rates in Australia for Selected Stallions over a 13 year period\*



\*Data compiled from the HRA database. Considering only Stallions that served more than 20 mares within Australia with frozen semen over the 13 year period.

Table 3: Estimated Conception Rates for Selected Stallions available in Australia via Imported Frozen Semen over multiple seasons (1996/97 to 2013/14)\*



\*Data compiled from the HRA database, using Registered International Semen Transport Lists for seasons 1996/97 to 2013/14 inclusive.

Frozen Semen Service figures were determined by exclusion of data from Imported Stallions that were domiciled in New Zealand for the season. It should be recognised that this is an estimate only. Additionally, the data does not include the minority of services from those stallions that were domiciled in either New Zealand or Australia while being available to Australian Breeders via frozen semen, nor does it include services with frozen semen from deceased stallions.

## 5. IDENTIFYING THE SKILLS AND KNOWLEDGE ENHANCEMENTS REQUIRED

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- There is no accepted standard or protocol for semen quality testing of imported (or domestic) frozen semen, contributing to an in-consistent semen product.

### **Opportunities:**

- Continued improvement of education, training and accreditation programs will aid in assisting improved service provider and mare management outcomes, and reduction of cost to the Standardbred Racing Industry through breeding management inefficiency.
- The use of frozen semen will contribute to further expansion of the gene-pool through increased utilization of current world-performers and bloodlines.
- The use of frozen semen will contribute to the continued development of the Australian-bred Standardbred racehorse, so as to be recognisable and commercially valuable in an international (global) market.
- The use of frozen semen may provide breeding options to those breeders based in geographic locations where delivery of a chilled semen product is not viable or reliable within the timeframe demanded by the lifespan of the chilled semen product or where such delivery is not economically feasible.

### **Threats:**

- Inefficient breeding management in regard to the use of frozen semen may result in the loss of breeders to the industry through dissatisfaction. A negative breeding experience, especially when emotionally or fiscally expensive, has the potential to result in the loss of that breeder to the Australian Breeding Industry.
- The current trend of declining foaling numbers has immediate and ongoing ramifications for the racing industry and has the potential for significant economic impact through a reduction in racing product and wagering turn-over. Improvement in conception rates will have a positive effect on live foal numbers.
- The potential loss to the Australian breeding industry of high profile stallions due to owner reluctance or refusal to make such valuable horses physically available for service in Australia due to a perceived or actual risk of injury, sickness or disease. The use of frozen semen accommodates such stallion owners and allows those sires to remain accessible to Australian breeders.

**5. IDENTIFYING THE SKILLS AND KNOWLEDGE ENHANCEMENTS REQUIRED**

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## 6. THE INTERNATIONAL EXPERIENCE

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In June of 2015 the Fellow travelled to Sweden to observe and discuss the use of frozen semen in an Equine breeding program.

The breeding season in Sweden usually starts the 1st of March and continues until the middle of August. All trotting horses registered with the Swedish Trotting Association are officially born on the 1st of January. Stallions may cover a limited book of mares - 150 mares for those stallions using artificial insemination or 100 mares if using natural breeding. However a stallion may also serve additional mares that are domiciled outside Sweden using transported chilled or frozen semen if suitably (EU) approved. Swedish regulations state all artificial breeding must be performed by a licenced Veterinarian educated for A.I. in the horse (1 week course) or a licenced A.I. Technician educated for A.I. in the horse (2 week course) provided that the mare had been examined by a licenced Veterinarian.

The destinations visited by the Fellow were:

- Svensk Transport - Swedish Trotting Association)
- Menhammar Stud - Sweden's largest Standardbred Stud.
- Erikssunds Stud Farm - Standardbred Stud Farm
- Skråmsta Stud - Standardbred Stud Farm
- Lövsta Seminstation – Performance horse stud, primarily warmbloods
- Kerstin Darenius – Equine Reproduction Specialist Veterinarian and Swedish Affiliate Laboratory of Select Breeder Services.

### Svensk Travsport (Swedish Trotting Association), Sweden

**Contact:** Christina Olsson, Director of Breeding for the Swedish Trotting Association

Svensk Travsport is the National Controlling Body for Harness Racing in Sweden. Part of Svensk Travsport's responsibilities include:

- Securing a quality product at the racetrack. The quality of the horses that compete have a direct impact on the development of the sport. Furthermore, in the interest of the future development of the sport it is important that the Swedish Trotter is competitive internationally.
- Maintaining a register of all horses working in the racing industry and to collect and analyse performance data pertaining to those horses to build a data bank of information constantly available for the evaluation of the breeding profiles of current and prospective Trotting sires.

Breeding Management was previously controlled by the state and stallions were judged to common standards applied across the racing breeds, Standardbred and Thoroughbred. Under the current model of assessment, the Trotting Stallion is given a predicted breeding valuation based on research and the demonstrated heritability of many individual traits common to the elite trotting race horse. Of a maximum 100 points each stallion is rated according to

1. Racing results (50 points)
2. Pedigree (40 points)
3. Soundness (10 points), as assessed by a veterinary check-up and X-ray examination

Installed in 2010, this program has amassed a large volume of information to contribute to the ever improving picture of what constitutes an elite equine athlete.

Conformation is generally considered an indirect method of estimating an individual's potential for athletic ability and the relationship between conformation and the limitations of the horse's performance is something that has generated much discussion. In the assessment of the Stallion, conformation is described but is not involved in the calculation of the expected breeding value.

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The volume of information provided for the benefit of the breeder by Svensk Travsport both through their website (travsport.se) and the annually published Stallion Guide (Varmblodiga Travhingstar) is comprehensive. The Stallion Guide presents statistical and physical information relative to each registered stallion, including pedigree, race performance results, veterinary assessment and fertility data. The fertility data comprises seasonal and first cycle conception rates for individual stallions and also conception rates for insemination methods – natural service and A.I. using fresh, chilled or frozen semen.



*Jog work at Solvalla, Sweden's largest Harness Racing track and home of the Elitloppet*

### Menhammar Stud, Sweden

**Contacts:** Johan Hellander, Studmaster and Chief Veterinarian  
Miguel Ferrer, Associate Veterinarian

#### Stud Overview

Menhammar Stuteri is Sweden's largest and most successful Standardbred Breeding Farm in terms of progeny stakes earnings. Owned by the Wallenius-Kleberg family, the stud can trace its origins back to the 1900's. Over the last 20 years the Stud has continued to expand its operation and today they own almost 300 horses including broodmares, yearlings, racehorses and stallions at stud. The horses are spread between the main farm in Sweden (Menhammar) and Stoner Creek Stud in Kentucky, USA.

In 2015 Menhammar stood 11 stallions at stud in Sweden, including successful sires from their own breeding program such as 'Maharajah' and 'From Above'. In addition to their own mares, Menhammar take in approximately 200 'outside' mares each breeding season to be bred to their stallions.

The stud employs approximately 25 people all year round. Almost all staff live on the property. The stud is managed by Chief Veterinarian, Johan Hellander. Dr Hellander is supported during the breeding season by a second resident Stud Veterinarian, Miguel Ferrer.

#### Mare Management

##### Mare Selection

Ideally, mares with poor fertility would not be included in a frozen semen breeding program. However economics have a strong influence. For instance, sometimes a minimum number of mares may be required to qualify for a stallion credit (discounted service fee rate) and so less suitable mares would be included to make up the required number.

##### Mare Examination

The mare is examined, in most cases every other day, to determine when she is ready for breeding. Detailed records were kept for each examination noting:

1. Tone and size of the uterus,
2. Size and texture of follicles and ovaries,
3. Tone of the cervix
4. Presence of uterine oedema and/or fluid
5. Presence of an ovulation or corpus luteum (and number of)

The early pregnancy exam is performed at day 16 - 17, unless a double ovulation was noted which would indicate the likelihood of the mare producing multiple pregnancies. In these mares the pregnancy exam would be performed at day 14 - 15 post service as the success rate is high for twin reduction (twin crushing) if performed early. The presence and number of corpus lutea are noted. The 28 day (mid) pregnancy check is considered to be most important. Again the mare is checked to be carrying a single fetus, but the presence of a fetal heartbeat and gross normal development is checked and recorded. A final pregnancy check is performed at day 45 at which time the service fee is generated.

The mare examination procedure was common amongst all studs and service providers.

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Examination of the mare

### Induction of ovulation

Mares for frozen semen service were programmed for ovulation using hCG (Chorulon) exclusively. This produced very reliable results, providing the mare had no history of endometritis. When the mare was determined to be sufficiently advanced in her oestrus cycle to be predictably responsive, she was administered the ovulation inducing hormone hCG. The size of the follicle alone was not considered sufficient to warrant administration of hCG to induce ovulation, the presence of uterine oedema and a softening cervix were also important indicators.

*Note: hCG is the only ovulation induction drug available in Sweden, GnRH (Ovuplant, Deslorelin) is not available for use.*

*"We give the mares hCG to program for ovulation. It works best for us to give the hCG either very first thing in the morning, or very late at night. That way you are looking to breed the mare during the day when there are people around. We start scanning the mare at 24 hours (after administration of hCG). Maybe you will miss 1 by starting then, maybe 1 in 100 will ovulate earlier. Then you have to follow the mare. Experience will tell you if she is not ready, if the follicle isn't soft or a bit painful, or if there is too much oedema. Then maybe you can extend the time to the next scan out a bit longer – and if she is close then you can scan a bit sooner (than 6 hours)". (Miguel Ferrer, Associate Veterinarian)*

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### **Preparation of the mare for service**

In readiness for insemination the mare, having been suitably restrained in the breeding crush, must be thoroughly cleaned. This includes:

1. The tail is wrapped with a bandage or tail wrap and tied up or to the side to prevent hair from contaminating the cleaned area
2. The mare's vulva and anus are carefully and gently scrubbed with a low pH soap and water to remove all dirt and debris
3. The cleaned area is dried thoroughly with paper towel.

The cleaning of the mare is an essential part of the routine to protect the mare from infection by reducing contamination of the reproductive tract with bacteria or debris. High standards of hygiene should always be in place for the protection of the mare. Poor hygiene will contribute to a reduced pregnancy rate.

The mare preparation procedure was common amongst all studs.



*The mare is cleaned in preparation for service*

### **Thawing the Frozen Semen**

1. A small goblet is pre-cooled in liquid nitrogen. The required number of straws are transferred from the semen storage tank into the goblet.
2. The goblet is taken to the waterbath. The temperature of the water is 37.C, and the bath must contain sufficient volume that the water temperature will not be significantly altered by the addition of the frozen straws. All straws are emptied into the waterbath at the same time (making sure all the liquid nitrogen has been removed before emptying in the straws so as not to tip liquid nitrogen into the waterbath).

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3. After 30 - 60 seconds the straws are removed from the bath and properly dried with paper towel. When thawing at 37 degrees centigrade the straws can be in the water for longer than 30 seconds.
  4. Invert the straws and 'flick' to move the air bubble to the sealed (ball bearing) end. Cut off the sealed end and wrap the straws together in paper towel to protect from sunlight (Sperm cells are sensitive to sunlight but not to artificial light).
  5. The semen is microscopically assessed for post-thaw motility.
- The straws are now ready for insemination.

### **Insemination of the Mare**

The mare is inseminated using deep horn insemination technique, using an intra uterine insemination (IUI) pipette and stylus.

1. The IUI pipette is introduced through the cervix.
2. Via rectal palpation the pipette tip is guided to the tip of the uterine horn on the same side as the ovary with the ovulation.
3. With the pipette remaining in place, the straws are introduced into the pipette and emptied using the metal stylus one at a time.
4. When the last straw has been emptied, the pipette is removed.
5. All information on the straw (stallion name, year of production and batch number, freezing centre) as well as the number of straws used and estimated post-thaw semen motility is recorded on the mares file.

It is preferable to use the least amount of semen possible without compromising the pregnancy rate. The insemination dose used then is dependent on:

- a) Sperm concentration
- b) Post thaw semen motility
- c) The individual stallion's fertility (established pregnancy rate).

*"We use a deep horn insemination technique always. And sometimes with a reduced straw number, less than a dose. But you need to know the stallion then, know what his fertility is. Some stallions it doesn't matter if you have 8 or 800 straws, they won't go in foal. Other stallions get good results with less than a dose. But you have to know the semen. The timing of the mare will influence that too. If you are breeding very close to ovulation then you can use a smaller number of straws and still get good pregnancy results.*

*I think that if the semen is very good then it doesn't matter what method you use, the mares will go in foal. When the semen is not quite as good, then you see the differences between the bases, between Veterinarians, in the results. Those that are prepared to work harder, to be a bit more thorough will get better results. Or if they have more skill or experience they will get better results". (Miguel Ferrer, Associate Veterinarian)*

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*Wet mares waiting in the crushes for insemination*

### Lövsta Seminstation, Sweden

**Contacts:** Görel Nyman, Stud Veterinarian  
Florian Darcourt, Stallion Manager  
Malin Cohlin, Breeding Manager  
Nina Känsälä, Stud Groom

### Stud Overview

Lövsta Seminstation opened in 2010, and is today one of the leading Warmblood breeding stations in Sweden. In 2013 Lövsta inaugurated both a new insemination-hall (breeding barn) and a new stallion stable including a stallion collection room and laboratory. As a natural part of the vision to develop Swedish equestrian sport Lövsta has continued to focus on elite dressage and jumping stallions and by partnering with the leading players in Europe they are able to offer semen imports from several exciting bloodlines. 2014 saw the continued collaboration with the prominent European Paul Schockemöhle Stable.

Today Lövsta presents a comprehensive line-up of the modern Sporthorse bloodlines, available on farm via artificial insemination using fresh semen from their own stallions, using transported chilled semen from Swedish or non-Swedish based stallions or using frozen semen. Additionally their own stallions are available abroad by transported and frozen semen.

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Lövsta Seminstation employs six full-time staff including Reproduction Barn Manager Malin Cohin, and Stallion Manager Florian Darcourt. Stud Veterinarians, Lena Malmgren and Görel Nyman, reside a commutable distance from the stud and attend the stud daily during the breeding season.

### Mare Management

The mare population presenting at Lövsta was of low average fertility. Predominantly Warmblood mares, they were entering the breeding barn often at the end of extended competition careers and as older (maiden) mares. The average age of the maiden mare presenting for service barn was 11 year old as compared to an estimated average age for the maiden Standardbred mare of 7 years old.

Additionally the quality of the frozen semen supplied from abroad was often compromised both in quality and quantity and accompanied by little or no statistical fertility information.



*Mares wait in the yards beside the breeding barn*

### Mare selection

While the stud may recommend that a mare be unsuitable for breeding with frozen semen due to poor fertility, as suggested by her previous reproductive history or current status (older maiden or barren mare), the decision always remains with the mare owner.

*“We ask (the client) “do you really want a foal or do you want a foal after this or that stallion? Is it the foal or the stallion that is important?” (Nina Känsälä, Stud Groom)*

Communication with the owner is vital to ensure the expectations of achieving a pregnancy (and resultant foal) are reasonable.

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Embryo Transfer mares are not recommended for frozen semen service at Lövsta as they feel there is associated risk with the additional manipulation of the frozen semen embryo – that the frozen semen embryo is much more vulnerable than a normal embryo and the rate of resorption is much higher.

Lövsta does not breed mares on 'foal heat' with frozen semen.

### **Mare Treatment**

A uterine swab and culture is performed on all mares presenting for breeding with frozen semen. Swabbing reduces the number of unreasonable negative returns. A mare with a bacterial infection may show no visible symptoms but will often return a negative pregnancy test. The pre-service swab then will indicate the post-service treatment. Cultured bacteria is identified and where necessary an antibiotic sensitivity test is performed to indicate the specific anti-bacterial treatment.

Mares with pre or post-service uterine fluid are flushed repeatedly with sterile dilute sodium chloride (NaCl 9%) solution until the recovered fluid runs clear. Repeated low dose administration of oxytocin is also used for clearing fluid.

Lövsta is often presented with aged maiden mares. These mares often have fluid (in the uterus) and the cervix is long, "cigar-like" and remains tight even while the mare is in heat.

*"We wait and see how they react to insemination, but my experience is that we have to flush them. Commonly in these mares the cervix doesn't open sufficiently or for long enough for the mare to clean herself. So the fluid stays in the uterus. So that is the problem. They still have uterine contractions but the fluid can't be expelled because the cervix is tight. For these mares I don't like to give prostaglandins (to shorten the period of dioestrus and return the mare more quickly into the oestrus phase), I like to let them come in on their own. Their hormones are then more in balance". (Görel Nyman, Stud Veterinarian)*

### **Uterine cysts**

Older mares (maybe also in some young mares too but mostly increases with age) may have many cysts, small and large. Cysts will not influence conception but can affect the pregnancy rate in three ways:

1. Limit the movement of the embryo
2. Decrease the available space for implantation
3. Decrease available endometrial (uterine mucosal) wall available for placental attachment.

Mares with many or large cysts then may suffer pregnancy losses through disruption of implantation of the embryo or through placental insufficiency. Mares with very large or multiple cysts, if they are valuable enough, are ideal candidates for embryo transfer.

The position and size of cysts are recorded before insemination as the mare must be very carefully checked at the time of pregnancy testing to discriminate between a cyst and a pregnancy.

### **Insemination Protocol**

Ovulation is induced using hCG for all frozen semen services. This differs from those mares covered using A.I. with fresh or chilled semen who are generally served without induction of ovulation. The mare intended for a frozen semen service is scheduled for ovulation between noon and mid-afternoon when staff are available, so requiring administration of hCG at 12 -3am the day prior.

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Insemination with frozen semen is always on visualisation of ovulation. The mare is scanned every 6 hours until ovulation is observed. In the period surrounding perceived imminent ovulation the frequency of scanning may be reduced to every 2 hours so as to be inseminating as close as possible to ovulation.

*“Usually we only have a very small amount of semen, often only two straws. So we can only afford to inseminate once. If semen was readily available then we may use the timed ovulation method because it would be so much less time consuming and we wouldn't have to be scanning repeatedly”. (Görel Nyman, Stud Veterinarian)*

Insemination with frozen semen is always performed using deep horn insemination technique. The mare is always inseminated with a full dose.

Amongst the warmblood breeders, the purchase of semen – not the pregnancy - is common. Often the frozen semen is purchased by the mare owner with no accompanying information regarding concentration or fertility statistics.

*“With the frozen semen that we use from our own stallions we know how many millions of sperm is in every straw. But if you buy (the straws), rarely do you get any information. It is like a lottery, I don't like it...you pay everything for the straw with no guarantee. The owners can spend 2,000 euros and they have nothing, maybe 1 or 2 live sperm. And the stallion owner takes it all with no risk”. (Görel Nyman, Stud Veterinarian)*

### **Stallion Management**

Given that many of the stallions are actively competing at international level, and that managing a breeding and performance career concurrently is often unfeasible, semen from the majority of their stallions is collected and frozen during the autumn or winter. Lövsta employs the services of Kerstin Darenius (Select Breeders Services Sweden), to freeze semen from the stallions. The semen is handled and prepared according to SBS protocol.

Warmblood breeders in today's market have the option of purchasing frozen semen straws (commonly enough for 3 standard breeding doses) or upon a 60 day positive pregnancy test. Purchasing the semen, not the pregnancy, usually reduces the service fee by 50%

*“The frozen semen is sold by dose because you have no control over what is done with the frozen semen once it leaves. But many farms, particularly in Italy where two straws per dose is normal, they are using two straws per service (1/4 dose) and getting very good results. We freeze all our stallions at 8 straws per breeding dose. What the owner dose with it is out of our control.*

*For transported semen we ship at one billion mobile sperm cells per dose, that is almost twice the European standard (of 600 million). We always try to send a double dose. But as mare numbers are increasing there is pressure to make the day's orders, especially as the stallions are becoming more popular and are competing well. With our frozen semen the concentration is always the same.” (Florian Darcourt, Stallion Manager)*

Bacterial swabbing is performed monthly to assess bacterial growth.

*“The Vet that runs our freezing program is very particular and absolutely controls the quality of the frozen semen that we send out.” (Florian Darcourt, Stallion Manager)*

### Erikssunds Stuteri, Sweden

**Contacts:** Ceve & Cattis Linde, Stud Managers  
Johan Arneng, Swedish Stallion Manager (Stallzet)

#### Stud Overview

A historically significant Estate, Erikssund Farm was purchased in 1959 by Ellert Larsson to begin operation as a racehorse breeding and training complex. Recently the Larsson family has reduced their breeding involvement and Stallzet (Stable Zet), a professional breeding and training operation with stables in both Sweden and America, have moved their broodmares to Erikssund.

The Stud is managed by couple Ceve and Cattis Linde. In collaboration with Stallzet Stallion Manager Johan Arneng, Ceve and Cattis are responsible for all mare and stallion management. The Stud Veterinarian resides on the farm. With the involvement of Stallzet the mares at Erikssund are now almost exclusively privately (Stallzet) owned. Stallzet currently stand three stallions in Sweden, with additional stallions based in the U.S.A., including Hambletonian winner 'Trixtion'. Swedish based stallions are available via transported (chilled) semen. The American stallions are available in Sweden via frozen semen. Frozen semen breeds then account for approximately 50 per cent of services on the farm.

#### Mare management

##### Mare selection

There is no selection criteria for mares presenting for service. Whilst it is recognised that some mares have a significantly reduced chance of conceiving using frozen semen, the desired resultant foal as racing or future breeding stock is more important than an individual pregnancy from that mare.

Mares are not served on foal heat with frozen semen. There may, however, be exceptions made at the end of the season when time to get the mare in foal is running out.

##### Timing of insemination

The timing of insemination is crucial in achieving a high pregnancy rate with frozen semen.

*"You must have a good check on the mares, that is very important. If you have bad semen you need to be more intensive on the mares. In one way it is very good to have frozen semen because you always have the semen here in the tank ready when you need it. In that way frozen semen is very special. And it is what it is! You know what the concentration is and you know how good it is (or isn't). If the mares all go in foal then you can reduce the breeding dose if need be because you know what the semen quality is.*

*Our Vet lives here on the farm, so he is available to scan the mares when he needs to. The timing of the mare is very important. The most ideal, to give the best results, is to breed just before ovulation. But that is very difficult to tell. The skill of the vet is also important – but not just the vet, also the people that are caring for the horse, to know how she is." (Ceve Linde, Stud Manager)*

##### Semen Thawing Protocol

If the semen is accompanied with thawing instructions from the organisation supplying the semen then they should be followed.

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Otherwise, a bucket of water at 38.C is placed beside the semen storage tank. The straws are removed from the tank and dropped one at a time into the bucket. After the last straw enters the water the timer is started. After 30 seconds the straws are removed from the bucket and dried thoroughly.

### **Insemination Protocol**

The mare is administered hCG to induce ovulation. Then mare is then scanned at six hour intervals, beginning at 24 hours after administration of hCG. The mare is inseminated as soon as ovulation is observed.

Post-ovulation the mare is checked for fluid and flushed if necessary. But little flushing or treating with antibiotics is undertaken.

*“We always serve on ovulation. If we have plenty of semen then we use the old way, same as for chilled semen. We empty the (thawed) straws into a warmed syringe and inseminate through the cervix with a normal pipette. If we don't have so much semen then we use the deep horn technique and then we use only a half dose”. (Ceve Linde, Stud Manager)*

### **Stallion Management**

The management of the breeding stallion can be logistically very difficult – even more so when the stallion is concurrently breeding and racing.

*“Managing the stallion in the breeding shed can be very difficult. Sometimes there will be orders for the same mares again and again.....they order the semen and then 2 days later they order again, and the again in another 2 days time. It puts a lot of pressure on the stallion and on us. The first year for a stallion is very important. And some popular stallions take too many mares. When he starts the semen is good and the mares go in foal. But then there are problems when the mare numbers are high because of pressure to supply.*

*Frozen semen is very special, because you have it in the shed waiting for when the mare is ready. And it is what it is. You know the concentration and how good it is. If the mares all go in foal then you can reduce the breeding dose if you have to because you know what the semen quality is”. (Ceve Linde, Stud Manager)*

### Skråmsta Stuteri, Skramasta, Sweden

**Contact:** Carina Dahling Satrell, Stud Owner and Manager  
Cecilia Tideström, Stud Veterinarian

#### Stud Overview

Skråmsta stud farm is one of Sweden's oldest currently active breeding stud farms for trotting horses. Comprising of 210 hectares of pasture and arable land, the Stud is managed by experienced Studmaster and Propietor Carina Dahling Satrell.

The farm employs four people full-time and extra hands during the breeding season. Cecilia Tideström is the Stud Veterinarian and resides 40km from Skråmsta and visits the farm every second day or as necessary. Cecilia Tideström is an experienced Equine Reproduction Specialist, particularly with frozen semen.

Skråmsta Stud foals down approximately 40 mares each year. Inseminations are performed using fresh, transported and frozen semen. The mares at the stud are almost exclusively owned by outside interests and utilize either Skråmsta based stallions or stallions from other studs, both within and outside of Sweden.

In 2015 Skråmsta stood three commercial stallions, including the Elite stallion 'Scarlet Knight'. All stallions were available via fresh or transported semen.

#### Mare Management

##### Mare selection

If the stud considers the mare to be unsuitable for a frozen semen service they will strongly suggest that the owner consider a more suitable stallion choice. The mare should be judged as an individual - an older mare that has a foal every year, if she has no trouble foaling then may be considered worth trying. But always it is the owner's decision.

*"We can say that they are not likely to get a foal but if they want to try then we will. Usually we give them two tries and if they are not pregnant then the owner will say "I concede" and change the stallion". (Cecilia Tideström, Stud Veterinarian)*

##### Mare treatment

The stud is very conservative regarding antibiotic treatment.

*"We don't use any antibiotics and I never swab the mares. Naturally the stallion would have bacteria on his penis when he served the mares. The area on the mare that is most contaminated with bacteria is the anus and the vulva. So I don't mess with the system - if God thought it was going to be a problem he would have put the vagina in a different place! But we do treat them with oxytocin (if they need it), and we make sure the mare is really ready. That's why I often feel the cervix too before I decide whether or not to program her to serve (or to serve her). Because I want her to be very open, so that I can breed her only once and she can clean herself". (Cecilia Tideström, Stud Veterinarian)*

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### Semen Thawing Protocol

Three key points to the thawing process were stressed:

1. The waterbath to thaw the semen must be at 37-37.5 degrees centigrade.
2. The semen must be thoroughly dried after thawing and protected from the sunlight.
3. At Skråmsta, all equipment that comes into contact with the semen is warmed to 36 degrees centigrade.

*"We always use the same pattern and it must be quick. Some people like to take all the straws together and then into another room and then into the water. But I think it needs to be quick. We put the water bucket next to the tank and then the straws come straight out of the (liquid) nitrogen and into the water, one at a time. Then they must be dried and covered from the light and kept warm. Then we cut the plug end and invert the straws over a warmed tube. Cut the other ends off and remove all the semen. Suck it up into a warmed syringe and then very quickly it is put in the mare, deliver into the uterus using a normal pipette. We do this every time and they go in foal." (Cecilia Tideström, Stud Veterinarian)*



Preparing to thaw the frozen semen straws

### Insemination Protocol

Three insemination doses are generally always provided for each mare. The stud always uses a full dose for each insemination.

The mare is administered hCG to induce ovulation so that the expected time of ovulation is mid to late morning. The mare is scanned first thing in the morning (approximately 30 hours post administration of hCG), and then every 6 hours or less until ovulation is observed. The mare is inseminated as soon as ovulation is observed or when ovulation is deemed to be imminent.

The preparation of the mare and timing of insemination are very important.

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*“The experience of the person doing the breeding with frozen semen is absolutely very important. It is not always easy and you have to be very precise. The semen can be very variable from season to season and between collects. It is a very small amount and you don't know what you get. The mare is very important. A good mare with poor semen and they will go in foal. We always record what happens. That way if we know everything went perfect and the mare comes back not in foal then we can tell the owner that everything was perfect. If we were a little bit late then we know it was not all perfect and there may be a reason for the mare not to be in foal. It is important then to talk to the owner so they understand what is going on.” (Cecilia Tideström, Stud Veterinarian)*

### **Stud Fees**

The use of frozen semen in Sweden incurs three separate fee for the mare owner; the booking fee to the agent, the stud fee paid to the service provider that cares for the mare and the service fee. Almost all service fees are payable on live foal.

*“The booking fee is three times more than for stallions using transported/fresh A.I. In Sweden the owner pays a booking fee to the agent that imports the semen. Then they pay the stud that does the work to get the mare in foal and then when she foals they pay the service fee. If she doesn't go in foal they still have to pay the stud and the booking fee. So it is very expensive if they don't get the mare in foal. The booking fee is high because the import fees are high, and then there are futurity payments like Breeders Crown etc. If the stallion then only gets one or two mares it is a disaster because he doesn't make any money. I didn't understand why the booking fee was so high until I became an agent this year for an imported frozen semen stallion. The paperwork and the import/transport fees are so high.*

*The frozen semen comes from all over the place – Italy, France, USA ... Sometimes it is 8 straws per dose, sometimes 6 straws, sometimes 4. And there is never any paperwork to say why. What is the concentration per dose? How did they decide how many straws make up a breeding dose? If they provided us with this information then we would know, and then we could make better decisions.” (Carnia Dahling Satrell, Stud Owner and Manager)*

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### Dr Kerstin Darenius, SBS Sweden

Dr Darenius is an Equine Reproduction Specialist Veterinarian. She manages 'Caballa', her private practice, and is a member of Select Breeder's Services, operating as 'SBS Sweden'.

### Mare Management

#### Mare Selection

The mare should be suitable. A mare that has been bred for a season with frozen semen for a negative return should not be tried again with frozen semen the next year. Mares that have a poor reproductive history, that are difficult to get in foal, or are very young (regarded as an under-developed three year old or less) are generally avoided. Maiden mares are suitable, as are wet mares (mares with a foal at foot), if not too old. Mares with a first foal at foot can sometimes be difficult.

No mares are inseminated with frozen semen at foal heat.

#### Mare Treatment

All mares except maiden mares are swabbed before insemination. Preferably swabbing is performed one to two days before A.I. to allow enough time to culture. Bacterial culture is performed on site to allow a quicker turnaround time.

Bacterial infection is a common cause of pregnancy failure or resorption. Candida (yeast) infection typically causes resorption at 20 - 30 days.

Treatment of the mare following service, if necessary, is most often systemic. Rarely is antibiotic treatment administered intrauterine as it is felt this gives a better result without irritation to the uterus. An additional benefit is that the treatment period can then be extended without causing problem as the cervix closes. Where flushing is required, NaCl solution only is used if the mare is on systemic treatment. Occasionally the mare is treated only with flushing of the uterus, with the antibiotic dissolved in the saline solution (with no deposition of antibiotic in the uterus afterward).

#### Semen Thawing Protocol

Prepare the following:

- Water bath at 37.0 degrees centigrade
- Paper towel
- Scissors
- Timer

A goblet is cooled with liquid nitrogen. Wearing gloves, the straws are transferred from the semen storage tank into the goblet. The goblet can be then taken to the water bath and the straws placed into the bath all at once. Start the timer. After 30 - 60 seconds the straws can be removed from the water bath and dried with paper towel.

This method is for 1/2ml straws. At 37 degrees centigrade it doesn't matter if the straws stay in longer than 60 seconds, there is no danger to the semen. The big maxi straws (4 or 5mL straws) are thawed at 50+ degrees centigrade for 40 seconds. Then one must be very careful with time because if left in longer then the temperature is too high and it will kill the sperm. But at 37 degrees centigrade there is no danger to the sperm cells.

## 6. THE INTERNATIONAL EXPERIENCE

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If the straws are taken in water then they will lay flat on the bottom of the water bath – they should sit with around 45 degree angle in the bath with the plugged end low. These straws that lie flat should be discarded as the sperm cells may be damaged. Occasionally the straws will leak at the non-plugged end, especially if they are sealed and these straws need to be thawed held upright. Straws that have steel or plastic balls as sealing are much safer.

The straws are carefully wiped dry with paper, are counted and determined that all are frozen from the same stallion.

Once thawed the semen should be used as soon as possible. However, if the stallion or batch is used for the first time, the total and progressive motility and speed is examined, using a heated stage and a phase-contrast microscope.

### **Semen Evaluation**

One straw per dose (0.5 microlitres (ml)) used for semen evaluation in the following manner:

1. 10 ml is used for bacterial culture
2. 0.1 ml - diluted in 9.9 ml of water to measure concentration (using a Burker Chamber)
3. 0. 4ml - diluted x7 in warmed extender then assessed after 20 minutes recording:
  - » Total motile sperm (TM)
  - » Total progressively motile sperm (TPM)
  - » Speed of sperm movement.

This is the normal thawing procedure for SBS Affiliate Laboratories, many also evaluate the semen using a CASA (Computer Assisted Semen Analysis) system – the advantage of this is the removal of human error or interpretation, and that the result is repeatable.

All information is recorded on the mares' record, but also used to build a profile of the individual stallion.

### **Insemination Protocol**

To achieve good results (with frozen semen) it helps to have good quality semen as well as a good mare.

The semen needs to be of good quality. If the semen is excellent then the timing is not so important, the semen can wait in the mare quite a long time before ovulation.

Insemination is always with a full dose, never dividing doses. The exception is if using fixed time insemination when the dose may be split between the two inseminations.

*"It is in my interest to get the mare in foal, it is not my interest to save semen." (Dr Darenius, Equine Reproduction Specialist Veterinarian)*

Insemination is made using a Minitube IUI pipette, directed into the horn. There is a balance between how much is gained from placement of the pipette (to deposit the sperm right at the tip of the uterine horn) and how much irritation is caused. If the sperm is fragile, or motility is very poor then placement of the pipette is important, but with normal semen it is not. If Maxistraws (5 mL straws) are used, the semen is transferred to a 5 ml syringe and insemination is with a Minitube IUI pipette with an inner catheter. This decreases semen loss in the pipette.

Dr Darenius will often not induce ovulation in the mares scheduled for frozen semen service, instead allowing them to progress naturally without manipulation. However, these are often mares with history of being difficult to get in foal and this approach requires that she scan the mares at eight hour intervals often for many days. Near ovulation the intervals between examinations may be shorter (four hours).

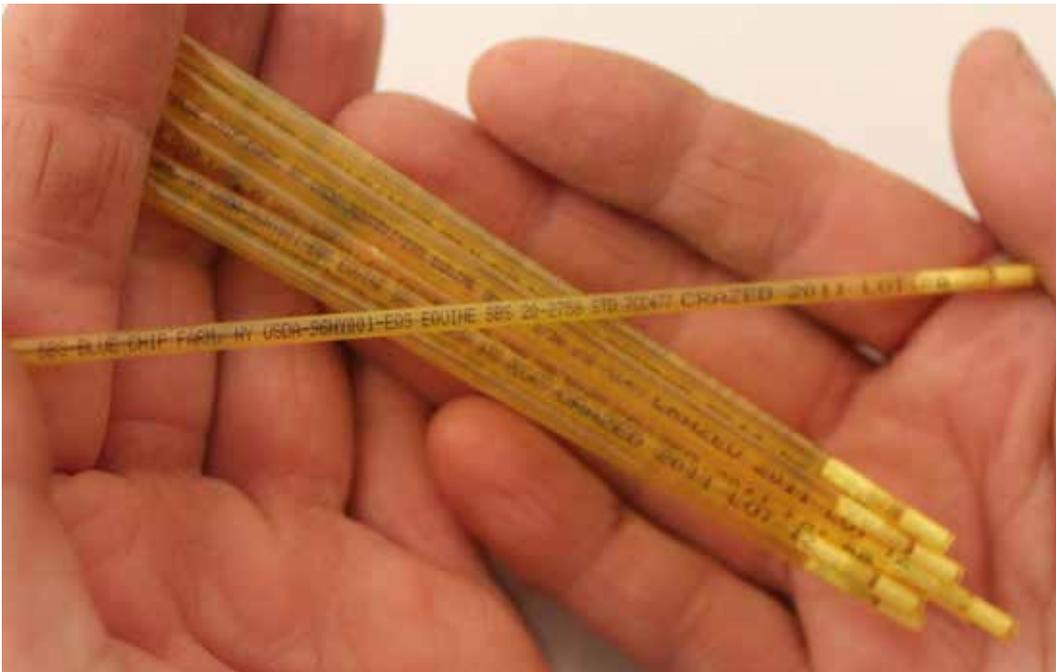
## 6. THE INTERNATIONAL EXPERIENCE

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At the time of insemination, all details relating to the straws are recorded on the mares file including:

- date of service
- year of production and batch number
- number of straws inseminated
- post thaw semen analysis results.

Individual straws are printed with information including: the stallions name; registration number and breed; and, the date of production. This information is vital for identification of the correct stallion for service. Additional information should also be printed on the straw such as the company performing the freeze, and the antibiotic used in the freezing extender.



*Detailed information is printed on each straw*

Additionally, Dr Darenus stores the empty straws, tagged with the date of service and mares name as a physical record should there be any future dispute. The empty straws should be stored for at least five years.

### **The Mobile Semen Freezing Lab**

The mobile Freezing Lab is a condensed version of the semen laboratory – it is fitted with all the equipment to process and evaluate frozen semen. Darenus' mobile lab is also EU approved to freeze semen for export, but only on EU-approved AI-stations (stud farms).

The mobile lab allows the laboratory to travel to the stallion and is particularly useful in the case of the competing stallion, where collecting for freezing would otherwise be a costly interruption to the training or competition schedule. It allows the stallion to continue his daily routine with minimal disturbance.

## 6. THE INTERNATIONAL EXPERIENCE

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*The Mobile Semen Processing Lab*

### **Quarantine Requirements**

Frozen semen is a biological product and so must be demonstrated to be free of transferable disease or infection.

Stallions engaged in a semen collection program for export must be:

- Housed in an appropriate quarantine approved facility. Contact with non-quarantined animals is prevented and strict hygiene and disinfection procedures are followed.
- Routinely monitored for disease status. This includes regular blood samples and genital swabs to confirm absence of transferable disease. Additionally bacterial culture of semen samples are performed for individual collects.

EU export guidelines indicate the specific tests required. Stallions housed under quarantine conditions would require testing prior to freezing. Those stallions that move into and out of the quarantine centre, such as for competition or training, require testing both before and after the freezing period.

The frozen semen must then be stored in conditions that maintain the integrity of the product. This includes storage of the straws and also of the liquid nitrogen holding tank in an environment free of potential contaminants. For example, storing the holding tank in a room containing a second holding tank containing untested and so potentially contaminated semen would violate the quarantine conditions and would prevent that tank and its contents from being able to pass through Australian quarantine.



## 7. KNOWLEDGE TRANSFER: APPLYING THE OUTCOMES

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Reproductive biology is the same from one country to another; the way in which frozen semen was used in Swedish horse breeding was similar to practices used in Australia.

Across the study destinations the Fellow observed a common sentiment - to achieve good pregnancy result with frozen semen requires:

1. **A skilled and experienced inseminator.** The more skilled the inseminator, the higher the conception rate will be. The use of frozen semen requires more intensive management of the mare, often at a time in the breeding season when the workload is already very high. To achieve a high pregnancy rate requires precise timing of the mare for insemination. The timing of insemination in relation to ovulation is crucial. Because of the reduced lifespan of the thawed frozen sperm in the reproductive tract of the mare, the mare must be inseminated within the window of 12 hours prior to or six hours following ovulation.<sup>1</sup> The ideal timing of insemination is immediately prior to ovulation, however this can be very difficult to accurately predict. For this reason, the most inseminators will serve the mare as soon as they observe that the mare has ovulated. Examination of the mare at six hour intervals ensures that the insemination is always within six hours of the mare ovulating. Additionally, while the lifespan of the semen once frozen is supposedly indefinite under correct storage conditions, the semen thawing procedure must be conducted with sensitivity to avoid damage to the sperm cells.
2. **A quality semen product.** Semen quality has a significant effect on conception rates particularly amongst less experienced inseminators. A highly fertile stallion will achieve good conception rates regardless of the manner of insemination. Frozen semen quality was observed to be highly variable, both between individual stallions and often between individual collects.
3. **Selection of a suitable mare candidate.** Mares considered most suitable for a frozen semen breeding program are maiden mares three - eight years old, wet mares under 18 years of age and mares that have not been served no more than two seasons ago. That mare then should be well prepared for service and monitored post-insemination for adverse inflammatory reaction (post-service oedema or delayed uterine fluid clearance) and treated as required. Control of the uterine environment post-service will improve the likelihood of achieving a pregnancy.

There is no doubt that excluding those mares that carry a compromised breeding history will improve frozen semen conception rates. However it is important to remember that the decision regarding whether or not to nominate a mare for a frozen semen service (providing that to do so does not have a negative impact on the welfare of the horse) ultimately rests with the mare owner. Often an owner will pursue a pregnancy via frozen semen from an unsuitable mare candidate because of the perceived genetic or commercial value of the desired progeny. In such cases, honest and direct communication between the mare owner and the inseminator is essential to produce a satisfactory outcome for that owner. The inseminator has the right to refuse to take on a mare they consider highly unsuitable or unlikely to achieve a pregnancy.

In the Harness Breeding Industry inseminators are generally supplied frozen semen of reasonable quality and quantity to maintain acceptable pregnancy rates. Under the existing practice of payment on confirmation of a 45 day pregnancy or the delivery of a live foal, the incentive is for the stallion owner to provide a semen product of good quality with regard to both sperm concentration and post thaw integrity - if a pregnancy is not achieved, then the service fee is not generated, while the stallion owner bears the cost of semen production. However, the semen controller is also under pressure to conserve semen, to logistically manage semen distribution with a usually limited seasonal supply. It is then in the interest of the semen controller (Stallion Manager) to allocate semen preferentially to inseminators that are the most likely to achieve and maintain pregnancies with frozen semen.

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<sup>1</sup> Avanzi, B.R., Ramos Rdos, S., Araujo, G.H., Fioratti, E.G., Trinca, L.A., Dell'Aqua, J.A. Jr., Melo E Oña, C.M., Zahn, F.S., Martin, I., Alvarenga, M.A., & Papa, F.O., (2015), 'Fixed-time insemination with frozen semen in mares: is it suitable for poorly fertile stallions?', *Theriogenology*, 83(9): 1389-93, June 2015.

## 7. KNOWLEDGE TRANSFER: APPLYING THE OUTCOMES

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Fixed-time insemination protocol for the use of frozen semen has been recorded in current literature as producing conception rates comparable to those using chilled semen.<sup>2</sup> This protocol also has the potential to reduce cost to the breeder while maintaining an acceptable conception rate if implemented properly, particularly in Australia to the breeder that maintains a broodmare population at their own premises, is certified in equine A.I., and uses a Veterinarian on a per visit cost basis. The procedure would be conducted as follows:

1. Day 1 - the veterinarian overseeing the program initiates induction of ovulation by administration of the ovulatory agent.
2. Day 2 - the mare is inseminated at 24hours by the breeder
3. Day 3 – the mare is inseminated again at 40hours by the breeder (inseminator).
4. Day 3 - the mare is examined to confirm ovulation by the Veterinarian / Technician at 52 hours, 12 hours following the second insemination.

Using this technique ensures the mare is inseminated sometime within the window for optimal conception (12 hours prior to or six hours post ovulation) provided that the mare responds favourable to the ovulatory agent.

The advantage of this technique is that it reduces the need for repeat examinations by the Veterinarian, so avoids the additional cost of the after-hours call out. It also reduces the work load of the Reproductive Specialist Veterinarian at a time when he is experiencing high demands, and emergency calls will take precedent over routine breeding engagements. Providing an effective working relationship and good communication are maintained, this protocol could offer significant labour savings while still maintaining acceptable pregnancy results. It then provides an opportunity for those mares be bred at “home” to be covered with frozen semen as the frozen semen mare can then be managed in a similar fashion to the mare served with transported chilled semen.

The major impediment to this methodology being accepted is the availability of sufficient quantity of semen. This method requires a minimum of two doses of semen per cycle, providing that the mare ovulates as predicted. The commercial management of a frozen semen stallion is an exercise in logistics. The quantity of semen available is almost without exception limited and the use of more than one breeding dose per cycle is difficult to justify.

Across all destinations the fellow visited in Sweden the reason given for not employing the fixed time insemination method was due to insufficient semen being available.

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<sup>2</sup> Crowe, C.A.M., Ravenhill, P.J., Hepburn, R.J., & Shepherd, C.H., (2008), 'A retrospective study of artificial insemination of 251 mares using chilled and fixed-time frozen-thawed semen', *Equine Vet.J.*, 40 (6): 572-76, September 2008.

## 8. RECOMMENDATIONS

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The cost to the owner of breeding with frozen semen was observed by the Fellow to be invariably higher than for A.I. with fresh or chilled semen, both in Sweden and in Australia. The additional cost associated with a frozen semen service is justifiable for the increased time and effort that it entails. However, the fiscal and /or emotional cost of failure to obtain a pregnancy - and ultimately a foal - through poor decision making, uninformed choices, or inefficient breeding management is negotiable.

To that end, and with the objective of improving reproductive efficiency in the Australian Standardbred Breeding Industry, the Fellow would make the following recommendations:

### **Provision of accessible information for the prospective or novice breeder**

Access to information regarding the use of frozen semen should be made available to the prospective or novice breeder.

The information should be presented in a manner that is easily understood and be free of specialist terminology. The information provided should also cater for the first time breeder to answer the question “What can I reasonably expect – what is the probability of achieving a pregnancy with my mare and what costs am I likely to incur?”.

There are several ways in which this information could be easily disseminated:

1. Harness Racing Australia have recently established Breeding Resources page. This site provides the ideal platform and is easily accessible by a large specific audience.
2. Harness Breeder's Associations can offer similar websites pages or social media links.
3. Public information sessions, to be conducted prior to the opening of the new breeding season.

The advantage of web based information display is the variety of ways in which information can be viewed. The opportunity for inclusion of video demonstration allows for a more engaging and perhaps more informative format.

### **Publication of National fertility results by the controlling body (HRA)**

Historically there was no distinction between frozen semen and chilled semen services, all were defined as transported semen. Consequently it was very difficult to establish trends of usage and conception rates, or to gauge whether pregnancy rates with frozen semen were comparable to those for chilled semen.

HRA has recently made changes to the way in which breeding data is collected and recorded, so as to be able to identify the mode of service: natural, chilled or frozen semen service. This will then allow for the building of a national database of information and presentation of fertility statistics (conception rates, breeding numbers and foaling percentages) specific to each mode of service. It will also facilitate direct comparison of pregnancy rates across the breeding season but also from year to year, providing an overview of the health and growth of the industry.

**Stallion statistics:** Publication of stallion fertility information, including seasonal conception rate and per cycle conception rates, could aid in improvement of conception rates by assisting the prospective breeder to make a stallion selection that provides the best chance of achieving a pregnancy. Every breeder should be aware of the fertility of the stallion they intend to breed with and should have access to information regarding semen quality.

**Inseminator / base information:** The availability to breeders of the conception rates for the use of chilled and frozen semen for inseminators would aid the breeder to select the service provider that best suits their needs. Under the current system of information collection, however, this information is not readily available. The Fellow suggests the state level Harness Breeders Associations provide a forum

## 8. RECOMMENDATIONS

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where it would be in the interest of those centres actively supporting the use of frozen semen to publish their conception rates for public viewing.

Traditionally the collection of information has centred on the stallion. The introduction and growth of transported semen facilitated the development of the commercial broodmare base, as well as increasing the number of mares serviced directly by Veterinarians or Specialist Breeding Technicians either at their prospective practices or on private properties.

With this in mind, the Fellow would suggest:

Collection of data at time of service to include a nominated inseminator. This would allow for generation of fertility statistics specific to the service provider. This would imply registration with the Harness Racing Board (state level) of the inseminator or associated Breeding Centre, and would be accompanied by an expectation of a minimum level of accreditation (Certificate of Equine Artificial Insemination).

The stallion and inseminator data-sets together, along with a realistic assessment of their mare, would provide the breeder sufficient information to select the best scenario for achieving a pregnancy from their mare. An older mare, or one with a less than favourable breeding history, may well be capable of achieving a pregnancy with frozen semen if the breeder is able to select a stallion with high fertility and a service provider with a strong record of results with frozen semen. Conversely, a breeder with a young productive mare may elect to utilize a stallion with lower fertility or a base that is perhaps less experienced but geographically – or financially – more suitable and still achieve a similar result.

Fertility results do need to be interpreted with some care, and it should be noted that one data set is not independent of the other. A breeding centre with a mare population that contains a high concentration of difficult or problem breeding mares may record a lower per cycle conception rate than other centres (although the overall (seasonal) conception rate however should be similar). Similarly, a stallion's pregnancy rate will be to some extent reflective of the skill of the inseminators using his semen.

### **Promotion of the Frozen Semen Product**

In order to promote the use of frozen semen as a powerful tool complimentary to our well established chilled semen program we must first recognise the success of the frozen semen product.

Many of the stallions standing via frozen semen are those sourced by astute breeders and studmasters because of their lineage and overseas performance record. These stallions however are often not recognised by the general racing public as they don't yet have an Australian profile.

Additionally the time lag between point of conception and race track performance is great and the number of mares served each season by a frozen semen stallion is small, usually less than 40. So there is often no recognition of those performers having been the result of a frozen semen service.

Highlighting the racetrack success of frozen semen progeny will go a long way to dispelling any myth or misconception about the value of frozen semen as a breeding tool. Public recognition of a horse having been the result of a frozen semen service would assist in attaining public acknowledgement of the success of frozen semen progeny.

Considering the Victorian Breeders 2014-15 Championship Awards, the list of frozen semen progeny was impressive:

- 'Our Waikiki Beach' ('Somebeachsomewhere') - 2 year old Pacing colt or gelding
- 'Charlie Runkle' ('Angus Hall') - 2 year old Trotting colt or gelding
- 'Menin Gate' ('Somebeachsomewhere') - 3 year old Pacing colt or gelding

## 8. RECOMMENDATIONS

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- 'Endsino' ('Angus Hall') - 3 year old Trotting filly
- 'Guaranteed' ('Artsplace') - Aged Pacing Horse



*Breeder's Crown Day - The Menin Gate Boys show their support*

'Somebeachsomewhere' is an interesting case in point. The stallion was first available to breeders in Australia in season 2010/11 via frozen semen. In each successive season he served mares with frozen semen, with the exception of season 2013/14 when he physically stood in Australia at Empire Stallions in Victoria. The resulting progeny of these chilled semen services are two year olds in season 2015/16. The Fellow has, however, noted on numerous occasions that public feeling is the racetrack success of the sire's progeny can be attributed to that chilled semen crop.

*"The early ones were the frozen semen horses and they weren't much good. Now we are seeing the horses (on the track) that he bred when he was here, that's why they are all winning ..."* (Public comment, Breeders Crown Final day, Tabcorp Park, Melton 2015)

This perception is not supported by the sire's progeny race performance results as tabulated below. Although no direct comparison can be made in this data set between the performance of chilled vs frozen semen progeny as there appears to be a correlation between the age of the horse and racetrack success (earnings), the results do refute the proposition that frozen semen progeny are inferior racetrack performers.

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### **'Somebeachsomewhere' (USA): Australian born progeny performance data\***

Season	Semen State	Number of foals	Number of winners	% Foals to Winners	Best Performer
2010/11	Frozen	19	13	68.42	'Wherelbelong' (\$410,877)
2011/12	Frozen	49	40	81.63	'Menin Gate' (\$502,335)
2012/13	Frozen	23	10	43.48	'Our Waikiki Beach' (\$665,221)
2013/14	Chilled	113	15	13.27	'Beach Goddess' (\$43,839)
2014/15	Frozen	85	-	-	not of racing age
2015/16	Frozen	18	-	-	not of racing age

\*Data collected from HRA Online database, current as of 28/6/2016

Furthermore, extending this data review to the two other stallions recording the highest number of frozen semen services in Australia within the last 15 year period, 'Life Sign' and 'Bettors Delight', produces a similar reflection.

### **'Bettors Delight' (USA): Australian Born Progeny Performance Data\*.**

Season Served	Semen State	Number of foals	Number of winners	% Foals to Winners	Highest Earner
2002/2003	Frozen	5	4	80.0	'Cyclone Betty' (\$68,186)
2003/2004	Frozen	3	2	66.7	'Vegas Bound' (\$357,628)
2004/2005	Frozen	19	10	52.6	'Emjayem Grand' (\$431,697)
2005/2006	Frozen	3	3	100.0	'Lynchman' (\$136,155)
2006/2007	Chilled	117	69	59.0	'Beautide' (\$2,183,132)
2007/2008	Chilled	80	51	63.8	'La Machane' (\$335,268)
2008/2009	Chilled	80	49	61.3	'Border Control' (\$515,435)
2009/2010	Chilled	56	31	55.4	'My Willow' (\$304,416)
2010/2011	Chilled	84	47	56.0	'Kept Under Wraps' (\$289,310)
2011/2012	Chilled	74	35	47.3	'Dodolicious' (\$337,773)
2012/2013	Chilled	82	17	20.7	'Weona Sizzler' (\$54,475)

\*Data collected from HRA Online database, current as of 28/6/2016

## 8. RECOMMENDATIONS

### 'Life Sign': Australian Born Progeny Performance Data\*

Season Foaled	Semen State	Number of foals	Number of winners	% Foals to Winners	Highest Earner
1998/1999	Frozen	1	1	100	Stella Fella (\$13,392)
1999/2000	Frozen	11	6	54.5	'Way Of Life' (\$240,910)
2000/2001	Frozen	6	4	66.7	'Lifes Rocky' (\$157,474)
2001/2002	Frozen	7	6	85.7	'Karoo Bronson' (\$26,611)
2002/2003	Frozen	7	4	57.1	'Real Life' (\$308,128)
2003/2004	Frozen	9	4	44.4	'Mister Anthony' (\$268,695)
2004/2005	Chilled	49	26	53.1	'Money Twitch' (\$563,777)
2005/2006	Chilled	217	84	38.7	'Crombie' (\$682,150)
2006/2007	Chilled	234	80	34.2	'Sign To Inverell' (\$370,212)
2007/2008	Chilled	218	76	34.9	'Red Salute' (\$206,469)
2008/2009	Chilled	210	63	30.0	'Bold Life' (\$175,120)
2009/2010	Chilled	91	26	28.6	'Vanesia' (\$47,778)
2010/2011	Chilled	48	9	18.8	'Mister Jogalong' (\$61,700)
2011/2012	Chilled	30	2	6.7	'Jogalong Stingray' (\$15,075)
2012/2013	Chilled	48	0	0	'Sign Of A Tyne' (\$2,690)
2013/2014	Chilled	19	0	0	Not of racing age

\*Data collected from HRA Online database, current as of 28/6/2016

The Fellow suggests that the mode of semen delivery (chilled or frozen) has no influence on the subsequent athletic potential of the resultant horse, that the genetic potential is unaltered.



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## 10. APPENDICES

**Appendix 1: Estimated Conception Rates calculated over multiple seasons (1996/97 to 2013/14)\* for Selected Stallions available in Australia via Imported Frozen Semen**

Name of Stallion	Number of mares served	Conception rate (%)
S J's Photo	91	82.4
Buvetier D'Aunou	29	82.8
Artsplace	109	70.6
Artiscape	121	71.1
Dream Vacation	65	72.3
CR Commando	91	74.7
Wall Street Banker	25	68.0
Sierra Kosmos	147	84.4
Muscles Yankee	76	76.3
Kadabra	21	76.2
Angus Hall	127	63.0
Camcracker	73	83.6
Muscle Mass	31	83.9
Well Said	78	78.2
Muscle Hill	35	71.4
Federal Flex	22	81.8
Majestic Son	37	75.7
Western Ideal	52	59.6
Western Hanover	37	73.0
Abercrombie	22	68.2
Astreos	169	62.1
Life Sign	135	76.3
Rocknroll Hanover	27	71.1
Grinfromeartoeat	51	56.9
Bettors Delight	55	60.0
Somebeachsomewhere	272	70.2
Average conception rate:		72.8

\*Data was compiled from the HRV database, using Registered International semen Transport Stallions Lists for seasons 1996/97 to 2013/14 inclusive.

Considering all imported semen data, frozen semen figures were estimated by excluding data from stallions that were domiciled in New Zealand for that season. It should be recognised that this is an estimate only. Additionally, the data does not include the minority of services from those stallions that were domiciled in either NZ or Australia while being available to Australian breeders via frozen semen, nor does it include those services from deceased stallions.

