

The International AI News from Minitüb

Sperm Notes

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Our knowledge - Your success

REF. 30100/2210

Foreword



Dear SpermNotes readers and friends of horse breeding,

Faster than one thinks, the old year (and even the decade) has passed and the New Year is already on course to engross us horse lovers, because: the breeding season has already begun. For us from Minitüb an exciting and special year is coming up, as we celebrate a round Anniversary: 40 years ago Minitüb was founded! It is a moment to pause in order to look back at an exciting time, but also to draw strength for the tasks that lie ahead.

But first of all we want to warmly recommend our product innovations to you on the following pages: they ensure that the daily lab procedure and collection routines go off reliably and properly without a hitch. In the field of ET, we also have come up with some new ideas for you.

It is a pleasure for us to help you with any questions that might arise regarding our products and applications. Please have a look at www.minitube.de, too: there, you do not only learn much more about our product range, but also find our new equine product catalogue for you to download, which we have reissued this year. Take your time, it is certainly worthwhile!

For comments and any ideas you are warmly welcome.
Write to: minitube@minitube.de or call us: +49 -8709-9229-0.

I wish you a successful year and a good breeding season and ... please consider Minitüb for your breeding supply needs in 2010!

Dr. Monika Esch
Product Management

Equine - Events 2010

| Dates | Event | Location |
|----------------|--|----------------------------|
| Feb 14 - 18 | Western Veterinary Conference | Las Vegas, USA, Stand 1269 |
| Feb 17 | Free Vitrification Webinar | |
| Jul 26 - 30 | International Symposium on Equine Reproduction | Lexington, USA |
| Aug 31 - Sep 2 | SFT/ACT Theriogenology Conference | Seattle, USA |
| Oct 21 - 23 | AETA Annual Meeting | Concord, USA |
| Dec 4-8 | AAEP Annual Convention | Baltimore, USA |

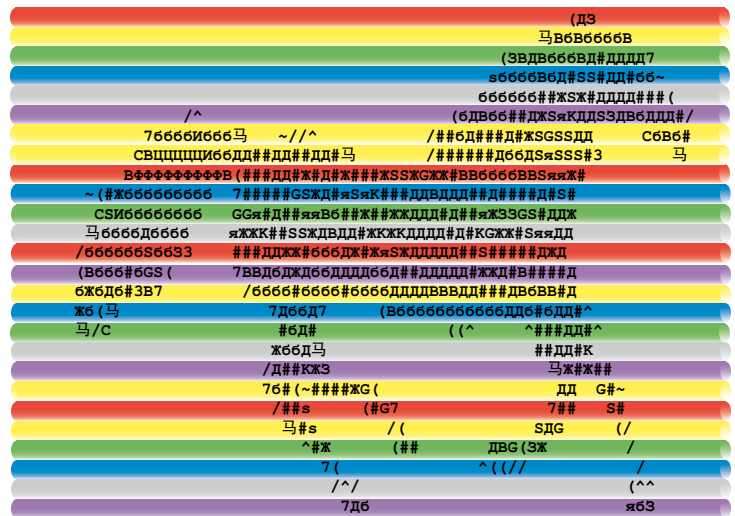
The EasyCoder 2.0

Not only is Minitüb celebrating an anniversary this year, also one of our best known and most popular products has an important anniversary: the EasyCoder!

Minitüb introduced the EasyCoder 10 years ago, just in time for the new millennium, as a new and unprecedented technology for the printing of straws for semen and embryos. Using thermal transfer printing, colour is applied onto the straw - a completely different technique to that of the conventional inkjet printers at this time and to older roller printers. 1300 small „dots“, strung on an area as wide as a straw, ensure that only the spots are heated and thus printed, which were previously entered into the control panel on the computer. This raises the possibility of integrating diverse characters, logos and barcodes into the imprint. The print itself is clean and razor sharp (300 DPI), unmatched by any other printing technology. Thus, even the labelling of the small ministraws can be read much more easily; furthermore the printing can neither be relieved nor smeared. And additionally: for the first time up to 3 lines could be printed on a straw, both 0.25 and 0.5 ml. With a capacity of 3,600 straws per hour, the EasyCoder therefore represented a user-friendly, powerful but yet relatively inexpensive alternative especially for the production of frozen stallion semen, as well as for medium and small bull semen laboratories.

The printer ensures a clean and odourless work as no ink or solvents are used. It requires very little maintenance and its functional design allows for easy and rapid conversion to the required straw sizes. In addition comes its compact design, which takes up little space in the lab.

Nothing has changed on the popularity of the EasyCoder until today: currently, it is in operation in over 100 labs all over the world - with a trend constantly increasing. On time for its 10th Anniversary, we sprang the EasyCoder's software a beauty treatment:



Various suggestions of EasyCoder users throughout the world have been included in the new software. The new EasyCoder 2.0 is now even easier to use than its predecessor; the software is more flexible, it includes more opportunities to adjust to the individual needs of a stud:

- sequential numbers can be printed on straws. The initial value as well as the step size (1 - 100) can be selected individually
- all data fields can now be renamed by the user and therefore adapted to the individual needs
- a search function allows you to browse the program for specific words or word parts
- in a daily log all print jobs can now be recorded. This log contains the master data of the animal, the number of printed straws and the print data of each day. This file can then be transferred into Excel
- an automatic backup with rotation on any path has been integrated: a daily backup and storage of all relevant data over a period of 1 month protects against accidental data loss
- all Windows language codes are now supported; printing of Cyrillic, Chinese and Japanese characters (Hiragana and Katakana) is now possible without difficulties

If you are in possession of an older version, you are nevertheless now able to profit from the new software: we offer a software upgrade - please contact your account manager at Minitüb!



REF. : 13038/0000

New Minitüb media for the cryopreservation of stallion semen

When regarding today's worldwide applied freezing protocols for stallion semen and the achieved results in motility and fertility, a significant variability can be observed in practice. You will find remarkable differences,

- when comparing the average results of different stallion studs or deep-freeze service providers
- when comparing the results achieved with different stallions; up to the point of absolute uselessness for cryopreservation
- when comparing different ejaculates of the same stallion.

All in all the freezing result of many ejaculates is not satisfying and a reduction of motility exceeding 50% after thawing compared with the original ejaculate is not unusual. This is on the one hand an indication of the understandably missing selection of fertility of the stallions, and on the other hand, however, an indication of the necessity to improve freezing protocols and to individualize them up to a certain degree. Amongst other things this results in the desire to provide a certain spectrum of cryopreservation media, in order to be able to satisfy the different requirements as well as possible. In addition it is necessary to formulate the media to be user-friendly; meaning to transport the media preferably without a cold chain and with only few preparation steps prior to the use in practice.

In recent years, several results in research and in cryopreservation practice indicated that the existing standard protocols for freezing should be revised. Better results were observed amongst others with the following changes:

1. Reduction of the egg yolk fraction without completely abandoning it

2. Reduction of the glycerine fraction, particularly individual adaptation to special stallions, depending on previous experiences

3. In addition to that, several laboratories achieved good results when the pre-diluted semen was slowly cooled to +5°C at first and only then the glycerine containing fraction was added. In that case the straw filling is also performed at +5°.

In cooperation with the UNIVERSITY OF VETERINARY MEDICINE HANNOVER, Minitüb performed several tests which dealt especially with semen of substandard original quality. Media with different amides (alternative cryoprotectiva; amongst others dimethylformamide) were applied as well as several combinations of different glycerine- and egg yolk fractions. The results of the media types containing amides did not meet with the expectations. In the same way those with a high fraction of egg yolk and/or glycerine achieved inferior results. Types with a low fraction of both components and without amides however achieved significantly better results and lead to the following conclusions:

- the optimum final concentration of egg yolk in a cryopreservation medium is 2%
- the glycerine concentration should not exceed 4%
- amides do not achieve an additional improvement under these preconditions.

On the basis of these and further scientific results, Minitüb has now developed a series of new media for the cryopreservation of stallion semen.

The product family EquiPro® CryoGuard™ is born.



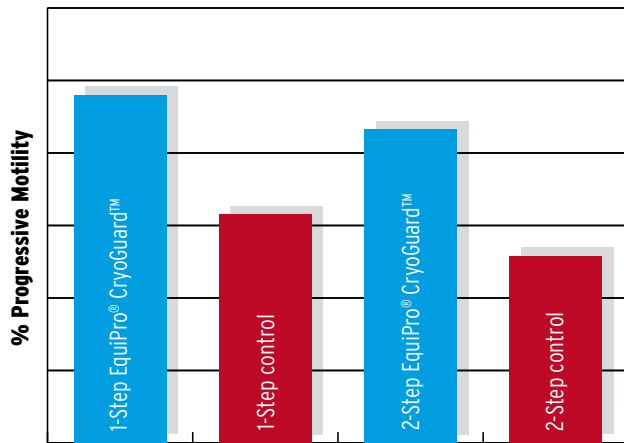
EquiPro® CryoGuard™ 1-Step
REF.: 13570/0410

EquiPro® CryoGuard™ 2-Step
REF.: 13570/0520

EquiPro® CryoGuard™ 2-Step
REF.: 13570/0510

EquiPro® CryoGuard™ 1-Step
REF.: 13570/0420

Graphic: Progressive motility of sperm cells after cryopreservation of stallion ejaculates with EquiPro® CryoGuard™ 1-Step and 2-Step.



In the context of these studies semen of several stallions was extended with the new EquiPro® CryoGuard™ media and cryopreserved. The results of EquiPro® CryoGuard™ 1-Step as well as 2-Step showed a higher motility after thawing compared to the control media.

EquiPro® CryoGuard™ is available as 1-Step and 2-Step type:

When using the 1-Step protocol, the glycerine containing cryopreservation medium is added to the semen before it is cooled down to +5°C. The 2-Step type means that the glycerine is only added after cooling to +5°C and all further preparation steps until cryopreservation are performed at +5°C. This procedure has proved itself advantageous for many stallions.

All EquiPro® CryoGuard™ media are provided by Minitüb in user friendly presentations. The media are either ready to use and simply composed, or a small quantity of egg yolk has to be added. It is not necessary to add pure water. Beyond that, the media are very stable and can be transported at ambient temperatures up to +25°C. For longer storage they should be kept in the refrigerator at approx. +5°C.

EquiPro® CryoGuard™ 1 Step

In the 1-Step-procedure the cryopreservation medium is added to the semen pellet after centrifugation at room temperature. Afterwards cooling to +5°C and freezing is performed. It comes as a ready to use medium which is available in two types:

a) EquiPro® CryoGuard™ 1-Step with egg yolk:

The extender is delivered in the form of 2 liquid components, the first containing 95 ml of medium and the second 5 ml of an egg yolk-glycerine mixture. The egg yolk Minitüb uses for this purpose comes from a controlled stock, and has been pasteurized and sterilised with gamma radiation. For use, both components must be mixed and then brought to the correct temperature at which the medium should be mixed with the semen.

REF. : [13570/0410](#)

b) EquiPro® CryoGuard™ 1-Step without egg yolk:

This type consists of only one liquid component, containing glycerine. The user adds 2 ml of freshly filtered egg yolk to the EquiPro® CryoGuard™. Afterwards the mixture is homogenized and brought to the desired temperature.

REF. : [13570/0420](#)

Protocol for semen processing with EquiPro® CryoGuard™ 1-Step:

The stallion ejaculate is extended 1+1 with liquid EquiPro™ and centrifuged. After removing the supernatant the semen pellet is re-suspended in EquiPro CryoGuard™ 1-Step to the calculated final volume. After dispensing into straws, cooling and freezing is performed.

EquiPro® CryoGuard™ 2-Step

This medium is delivered as two liquid fractions. Only the second fraction contains glycerine. The user performs the addition of the glycerine containing fraction in a second step, after the pre-diluted semen has been cooled to +5°C. Thus, the time period in which the semen is exposed to the glycerine is minimized. Again, this medium is available in two versions:

a) EquiPro® CryoGuard™ 2-Step with egg yolk:

This version consists of 3 fractions:

1. Fraction A: EquiPro™ for centrifugation and bloating of pellets
2. Fraction B: EquiPro® CryoGuard™: liquid medium, to which the egg yolk-glycerine component is added before using
3. Fraction C: Egg yolk-glycerine component

REF. : [13570/0510](#)

b) EquiPro® CryoGuard™ 2-Step, without egg yolk

This version consists of 2 fractions:

1. Fraction A: EquiPro® for centrifugation and bloating
2. Fraction B: EquiPro® CryoGuard™: the operator adds 4 ml of fresh egg yolk before using

REF. : [13570/0520](#)

Protocol for the semen processing with EquiPro® CryoGuard™ 2-Step:

1. Pre-dilute and centrifuge the semen with fraction A
2. Extract the supernatant and bloat the semen pellets with fraction A to half of the calculated final volume
3. Cool the pre-diluted ejaculate down to +5°C within 2,5 to 5 hours
4. Prepare the ready to use fraction B out of medium plus egg yolk-glycerine mixture (REF: 13570/0510) or egg yolk (REF: 13570/0520)
5. Precool fraction B and the filling- and sealing machine to +5°C
6. Add fraction B to the semen up to the final volume
7. Print the required straws
8. Fill and seal the straws at +5°C
9. Freeze them

Media and material for Vitrification and direct transfer of equine embryos

Cryopreservation of equine embryos allows owners to collect an embryo shortly after insemination and later to transfer the embryo for a convenient expected foaling date. It also facilitates the transport of embryos, especially international shipping, and it reduces costs and labour of synchronization of donor and recipient mares. In addition, the demand for parentage testing and pre-implantation diagnosis of equine embryos is increasing. The latter means that the embryos are examined in this very early stage for the presence of certain genetic constellations that can lead to unwanted diseases or characteristics.

The success of embryo cryopreservation is dependent on the developmental stage, size of the embryo and method. Smaller embryos collected 6.5 days after ovulation survive cryopreservation procedures much better than day-7 or day-8 embryos. Today there are 2 methods available: on the one hand the method of slow-cooling down to low temperatures which has been taken over from bovine ET and the Vitrification on the other hand. Whilst the slow-cooling is only possible with a specially developed device, the Vitrification technique does not require any expensive equipment:

Following washing of the embryo in EquiPRO™ holding medium, the embryo is placed in vitrification solutions. The embryo is then loaded into a 0.25 ml straw, which is sealed (e.g. via Ultraseal, REF.: 19500/2521) and placed into a cooled plastic goblet surrounded by liquid nitrogen for 1 minute. The goblet containing the straw is then plunged into liquid nitrogen.

Before transfer into the mare, the straw is taken out of the nitrogen and warmed at room temperature for 10 seconds before being

Table: Success rate of equine embryos transferred with the EquiPro® Vit-Kit™

| | |
|--|---|
| Mares flushed | 9 |
| Vitrified embryos | 8 |
| Transferred embryos | 8 |
| Pregnancies (Day 14) | 7 |
| Intentional pregnancy termination (Day 14) | 4 |
| Pregnancies (Day 120) | 3 |
| Live foals | 3 |

Quelle: ICB, Minitube

incubated in a water bath for an additional 10 seconds at 20-22°C. Subsequently, the embryo is directly transferred.

In 2008, as part of a clinical study at Minitube International Center for Biotechnology, a total of 9 mares were flushed on day 6.5 after ovulation and 8 excellent to good embryos were recovered. All 8 embryos were vitrified using the EquiPro® Vit-Kit™. The embryos were later warmed and transferred to synchronous recipient mares. At day 14, seven of the eight transferred embryos (87.5%) resulted in pregnancies. Because not all presumptive foals were needed, 4 recipient mares were randomly selected for intentional termination of pregnancy. The 3 remaining pregnancies developed normally and resulted in healthy foals.

Results from these studies demonstrate that the EquiPro® Vit-Kit™ can be used to successfully produce pregnancies, resulting in healthy live foals. Meanwhile, more results are available that document the successful transfer of embryos from which initially a biopsy was taken to determine the origin, specifically the presence of certain genes; then the embryos were vitrified using the EquiPro® Vit-Kit™, thawed and transferred. Here, 6 pregnancies resulted out of 8 transfers, 3 could still be confirmed on day 70. The vitrification with following transfer can thus successfully lead to pregnancies with biopsy treated embryos.



REF.: 19500/1200

New Products for Equine Embryo Transfer

For recovery:

Flushing Catheters made of silicone, CH 32 and 36, autoclavable, 100 ml balloon, sterilized,

Disposable Y-junction tubing for high flow rate flushing of equine embryos

Miniflush Filter for equine embryos with integrated petri dish and engraved grid for embryo search

REF. : [19010/2100](#)

EquiPro™: Media for successful ET

EquiPro™ recovery medium

complete formulation with antibiotics, available with BSA or PVA.

A Minitüb customer recently reported from his daily work: out of 17 positive uterus flushings with EquiPro™ recovery medium resulted 15 pregnancies. This represents a pregnancy rate of 88%!

- Available in 2.0 liter bags
- practical 3-way port for rapid delivery
- gas-tight package for longer shelf-life at room temperature
- intensive quality control, individual lot numbers for QC tracking
- Manufactured in Minitube's secure/dedicated clean room

with BSA
with PVA

REF. : [19982/2102](#)

REF. : [19982/2108](#)

EquiPro™ Holding Medium

zwitterion-buffered solution containing D-glucose, Napyruvate, 0.4% BSA, amino acids, Kanamycin and Gentamicin

- for in vivo and in vitro derived embryos at room temperature in atmospheric air
- for washing and rinsing embryos
- for loading embryos in straws for transfer
- packaged in 10ml tubes, 3 per package

REF. : [19982/2250](#)

EquiPro® Vit-Kit™

Media kit including disposable material for vitrification of equine embryos and direct transfer (see own article on the left side):

- kit includes all the necessary media and supplies to vitrify embryos.
- including set up, the vitrification process takes approximately 15 minutes.
- vitrification is safer for the embryo than conventional cryopreservation because it does not result in ice crystal formation that damages cell structures.
- vitrification requires no freezing machine; we recommend our Vit-Set for Vitrification and Cryopreservation of embryos and oocytes.

REF. : [19500/1200](#)

Set for vitrifying embryos or oocytes

The VitSet offers a practical set of tools for vitrification of oocytes and embryos in straws. Either OPS straws or normal 0.25 ml ministraws can be used for vitrification with the VitSet.

In the case of OPS straws, the VitSet offers a simple way to store the vitrified OPS within a larger straw which has previously been labelled. This larger straw can be sealed with a special Minitüb manual plier (REF: 19500/2500).

REF. : [19500/2000](#)





Minitüb celebrates its 40th anniversary

This year, there is a special occasion for Minitüb to celebrate: Exactly 40 years ago the company MINITÜB GmbH ABFÜLL-UND LABORTECHNIK was founded by Dr. Ludwig Simmet, a specialized Veterinarian for breeding hygiene and artificial insemination. Two years earlier, in 1968, he had developed as part of his veterinary work a novel method for the cryopreservation of bull semen and filed for patent. The „Minitüb System“ was the foundation of a success story, which led the company to the top of the world of reproduction technology within a few years, where it has established itself today as a specialized system provider in the fields of „Artificial Insemination“, „Embryo Transfer“ and “Assisted Biotechnologies”.

Meanwhile, the second generation has succeeded Dr. Ludwig Simmet: the four sons Florian, Ludwig, Christian and Rudolf Simmet now run the company, which employs today over 400 people in 9 offices worldwide, serving customers in over 100 countries all around the world.

Through our own research and development, the history of products used in equine artificial insemination and embryo transfer has been pushed significantly. As long ago as 1991, a complete catalogue was introduced for horse breeders and veterinarians. In addition to many other products, in particular our universal insemination pipettes for mares earned an international reputation: they are mentioned in many scientific and technical articles as well as reference books as the „gold standard“ for insemination of mares.



The catalogue for our horse products over the years



Image: Dr. Christian Simmet presenting the horse vagina Hannover (Catalogue 1998)

As equine semen is among the most valuable on earth, the extra attention is put on semen collection and processing. Here, Minitüb also makes an important contribution: machinery and equipment has been developed or adapted from other areas to meet the specific requirements of the horse. Today, Minitüb equips horse semen laboratories around the world: Kazakhstan, Qatar or Costa Rica are just a few countries where our products are delivered for equine reproduction and where the focus is set on High Tech by Minitüb.

Our team, including many veterinarians and technicians, will ensure that each of our clients receives the best possible care. With the establishment of subsidiaries in the major markets and partnership with a further 80 companies representing Minitüb in their countries, we ensure that our clients are provided the best possible service to enable successful operation.

It is our wish that you continue to put your trust in our products and that we will go on working as confidently and successfully as we do today.

We thank our many customers for 40 years of trust and look forward to continuing our cooperation!

Your Minitüb Team