



#### The international AI news from Minitube

# **Sperm Notes**

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### **Foreword**

Dear SpermNotes readers, Dear horse-breeding friends,

Another year has passed and a new breeding season has just started. With this year's SpermNotes we will inform you about events, new products and interesting methods in the field of equine reproduction.

Especially we take a closer look at our Sperm Vision Therio CASA system, but also at such exotic topics as reinforcement breeding.

This year there will be a lot of chances to meet us at conferences, fairs and courses. The data can be found right here on this page.

Have a nice time reading. By the way, previous issues can be found on our web page: www.minitube.com. We do hope you will visit this site. For comments and ideas you are welcome to send an email: minitube@ minitube.de or call us: +49 -8709-9229-0.

We wish you a good and successful horse breeding season!

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### **Events 2012**

| Period                     | Event  | Location               |
|----------------------------|--|------------------------|
| 19 21. January             | 7th International Conference on Equine Reproductive Medicine (ICERM) | Leipzig, Germany       |
| 03 05. February            | 18th SIVE International Congress                                     | Bologna, Italy         |
| 23. – 26. February         | Horse World Expo   | Pennsylvania (PA), USA |
| 29. July - 02. August      | 17th International Congress on Animal Reproduction (ICAR)            | Vancouver, Canada      |
| 29. August - 01. September | 16th ESDAR and EU-AI-Vets Meeting                                    | Dublin, Ireland        |
| 05 07. September           | 6th International Symposium on Stallion<br>Reproduction (ISSR)       | Vienna, Austria        |
| 13 16. November            | EUROTIER 2012  | Hannover, Germany      |

# The decisive factor is density -Sperm concentration measurements in stallions

To be successful in artificial insemination, one needs a certain amount of sperm of reasonable quality. The ejaculate is therefore analysed based on the following criteria

- Quantity (volume in ml)
- Concentration (million sperm per ml)
- Motility (percent moving sperm)
- Morphology (percentage of live sperm without abnormalities)

Minitube is now offering a new product for concentration measurement. The photometer SDM 1 has been specifically designed for sperm concentration measurement in animals and is produced in Germany. It measures turbidity with a 546 nm filter. A reliable LED light source guarantees constant light intensity. The result is shown in million (10<sup>6</sup>) spermatozoa per millilitre.

The SMD 1 works more accurate than other photometers, especially in the peripheral areas (very high or low concentration), thanks to the new measurement curve.



In addition to accuracy, Minitube gave also importance to simple and practical handling. Thus, baseline measurement for calibration of the system is carried out fully automatically. A separate calibration is not necessary in everyday use.

As a test sample for the determination of density, undiluted semen is used, so errors in preparation of semen samples are almost not existent.



#### Very convenient:

Apart from the power supply with a 12V transformer which is connected to the mains, the device can also be operated with batteries.

The micro-cuvettes have been developed especially for this photometer. They are precision manufactured. The length of the light path is 0.7 mm. They may be used at the same time as pipette, test tube and cuvette.



The round lid of the SDM 1 is fixed by means of magnets and can therefore be easily removed. The cuvette holder underneath the lid is removed by turning it around. Doing so one reaches, with only two movements and without any tools the appropriate parts. There can be cleaned easily with alcohol and a cotton swab.



### Minitube products in use - a case study

The Equine Reproduction center in Neustadt / Dosse has been a Minitube customer for many years and is almost exclusively equipped with Minitube devices. We would like to know how these perform in practice and so we asked the staff for some statements.

In Neustadt / Dosse there is also a major breeding stud in addition to the traditional stallion stud. 40 Elite and State premium mares form the breeding stock of Brandenburg's principal state stud. On the grounds of the main stud the Graf Lehndorff Institute for Equine Science is located. This institute is jointly operated by the Brandenburg State Stud Foundation Neustadt (Dosse) and the Vienna University of Veterinary Science.

The research focuses mainly on breeding and reproduction. Two stallion stations belonging to the stud (Neustadt and Krumke) are certified by the European Union as collection centers. The semen of their stallions is distributed throughout Europe for artificial insemination. Custom breeding by AI is offered at 13 additional breeding centers. Also frozen semen is available from selected performance sires. Currently, there are about 40 active stallions in the breeding stock. Among the best known and most frequented breeding stallions are Quarterback and his son Quadroneur, Samba Hit I, the sons of Levisto Z Levistano and Laspari and the Federal Championship finalist Lord Fantastic. For more information about the stud please visit www.sbhlg.org or www.pferdewissenschaften.at

#### Ice Cube 14 S with touch screen

"Practical in use and thanks to the monitor very well suited for demonstration purposes. Accurate programming of various freezing curves possible. The monitor provides the possibility to continuously control the progress (remaining time (stop time), actual and set temperature)."

**Warming cabinet/sterilizer UNE 600** "As warming cabinet and sterilizer at different temperatures in use. Easy to use. Partly also with time and day timer autonomous in use."



Water bath, 7 I

"Fast achievement of desired temperature after switching on. Very accurate temperature setting possible, easy to clean and disinfect (draining device)."

#### **MPP Uno filling machine**

"Easy to use, precise uniform filling of straws adjusted by vacuum. Very useful is the possibility of visual inspection during the working process of filling state and sealing of the straws."

> Sigma laboratory centrifuge "Works very well and reliably."

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**Bite belt for phantom for stallions** "The bite belt is very well accepted by the stallions and offers a sufficient surface and a good grip for the stallion. Depending on the stallion it is for sure highly stressed and has to be repaired sometimes."

**Low pressure steam sterilizer** "Adequate filling capacity, convenient and easy to use." **Leather cover for phantom for stallions** "The material is easy to clean and desinfect. Only the daily wet-cleaning during the season stresses the rivets on the closure strap."

#### **Photometer** "A very good device which we use a lot, both in routine as well as in training sessions."

**Phase contrast microscope Olympus CX 41** "Very good in quality and applicability, both in routine operations as well as in the training of students or trainees to insemination attendants."

#### Sperm Vision with PC and Camera, CASA Software

"We are working here without fluorescence device, this means that there are no live-deadanalyses possible, but for the computer assisted analysis of motility the system is ideal, both in routine operations as well as for research purposes. (Research projects on stallions at the Graf Lehndorff Institute are also based on the Sperm Vision CASA System)"

# **Recovery of Oocytes - Ovum Pick Up in the Mare**

Ultrasound-guided trans-vaginal oocyte aspiration provides an excellent non-invasive method for obtaining equine oocytes. In practice, this technique has superseded the more invasive laparoscopic techniques.

With the technique of OPU (Ovum Pick Up), oocytes (eggs) are picked up directly out of the follicle of an ovary, by means of an ultrasound device which is equipped with a needle. The ultrasound helps to visualize the ovary and guides the needle to the follicle which contains an oocyte. The needle is held in the vagina together with the probe of the ultrasound device by a probe holder. In addition a pump is needed which will facilitate the aspiration of the oocyte out of the follicle.

Horse breeders show more and more interest in applying so-called assisted reproductive technologies. Besides AI, the most established technology is Embryo Transfer. Despite of low embryo production rates because of little success in superovulating mares, ET is used more and more because it allows valuable mares and mares remaining in competition to produce foals.

## Why can ovum pick up be the better choice compared to Embryo collection and -transfer?

- The technique enables foals being born from mares which can not conceive
- Mares which don't get in heat
- Pregnant mares
- Mares with a very high genetic potential

Usually mares between 3 and 10 years of age, 400-500 kg and healthy udder are used in OPU-programs. However, many factors affect the recovery rate, such as the size of the follicle punctured and the stage of the cycle/pregnancy status of the mare.

#### **Ultrasound Equipment**

Various ultrasound machines have been used, usually with a sector transducer of various frequencies: most common is a 7.5 MHz probe.

#### **Mare Preparation and Restraint**

An adequate preparation includes the relaxation of the rectum and sedation of the mare just before the treatment. The mare should be restrained in a set of stocks/crush to limit her movement.

#### **Ovum Pick-Up Needle and Collection Systems**

A variety of needles of varying lengths and thickness have been used and either specially designed ovum pick-up needles or ordinary luer-lock disposable needles. There are basically two types of ovum pick-up needle systems: double or single lumen needles. The double lumen needle design allows both flushing and aspiration simultaneously.

The Minitube needle is a double lumen needle which includes a 16 gauge blunt inner cannula and a 12 gauge outer needle with a length of 24" (60 cm).





#### Needle Guidance System

A probe holder is needed in order to guide the needle into the follicle to be aspirated. On the ultrasound image on the monitor a needle/ biopsy guideline can be displayed which corresponds to the location and direction of the aspiration needle, making it possible to predict the direction of passage of the needle.



*Ultrasound Probe Holder and Needle Guide (REF.: 19009/4130) with Aloka Probe Retaining Insert (REF.: 19009/4140), for Convex-sector probe, 7.5 Hz (REF.: 23360/0502) or Sonosite Probe Retaining Insert (REF.: 19009/4141) for C-11 probe* 

#### **Puncture Technique**

The transducer within its holder is inserted into the vagina and positioned as far as possible to the left or right of the external os of the cervix. By rectal manipulation the ovary is positioned so that the follicle is in the line of the needle. Suction is immediately applied as soon as the tip of the needle has been seen to enter the follicle, and the follicle begins to collapse. Flushing of the collapsed follicle is performed; the needle is kept in place. If a second (or more) follicle is seen, this can be punctured and aspirated without withdrawing the needle from the ovary. This procedure should be repeated until all visible follicles have been aspirated.

With the double-lumen OPU needle with a bi-tubular connection between the needle and the vacuum system it is possible to do both, flushing and aspiration of follicles simultaneously. Modified Dulbecco's phosphate-buffered saline either with heparin, heparin and calf-serum or heparin, calf-serum and a penicillin-G/ streptomycin mixture is commonly used as flushing media.

#### Aspiration technique influences the recovery rate

Several aspects of the actual aspiration technique have been reported as influencing recovery rate. The use of a 12 gauge double-lumen needle rather than a 12 gauge single-lumen needle resulted in an increased recovery rate from pre-ovulatory follicles (84% versus 51%) (Cook et al 1993).

Different recovery rates reported in literature may also reflect the selection of different follicles for puncture: aspiration only of preovulatory follicles versus aspiration as long as more than 3 follicles are seen at the ultrasound examination of the ovaries.

Some form of suction is necessary to aspirate the follicles and to recover the fluid containing the oocyte. A suction pump with a variable vacuum pressure normally set at 200 mm Hg is commonly used.

Minitube offers an Equine Follicular Aspiration device with Miniflush & Media Warmer or alternatively a stand-alone aspiration pump.

Following or during collection into sterile plastic tubes, the follicular fluid is filtered. Later on, the oocytes are searched for under a stereo microscope at 120x magnification.

#### Techniques following OPU:

Fertilization of the oocytes is normally performed by ICSI (Intra Cytoplasmic Sperm Injection). Other techniques are Gamete

Intrafallopian Transfer (GIFT) and IVF (In Vitro Fertilization) in combination with IVM (In Vitro Maturation). Theses alternative techniques do not seem to give sufficiently good results.



## **Robust vapor freezing system for straws**

The freezing unit for 20 straws, 0,5 ml (REF.: 15043/0636) has been developed to fulfill the needs of labs who

 process frozen semen to a small extent with small numbers of stallions
 wish to perform a freeze trial, and don't want to spend too much

money for a computerized programmable freezer 3. need a transportable freezing system

The rack swims on the liquid nitrogen, and even when the LN2 level gets lower during the freezing process by natural evaporation, the distance

lower during the freezing process by natural evaporation, the distance between the straws and the liquid nitrogen is kept the same all the time.

An indication for the maximum/minimum level of liquid nitrogen assures the correct filling level of the stainless steel bath for every freezing cycle. Additionally the box is very well insulated for a low LN2 consumption.

When there are more than 20 straws to be frozen, a second freezing cycle can start immediately after the first one. For this purpose additional racks for straws are available (REF.: 5015043/0600) for storing the straws on racks in the refrigerator.



# EQUIPRO<sup>®</sup> Reinforcer Extender for stallion semen

Designed to boost the immediate capability & performance of spermatozoa during breeding.



Minitube's EQUIPRO® Reinforcer is the world's first stallion semen extender designed specifically for reinforcement breeding. The extender improves sperm transport in the uterus and optimizes survival of viable spermatozoa in the mares' reproductive tract.

EQUIPRO® Reinforcer has been clinically tested and is proven effective under field conditions. Minitube's Reinforcer extender is easily prepared using only a single 15 ml bottle for the breeding of each mare.

#### **Your Benefits**

- Clinically tested and proven under field conditions of reinforcement breeding
- Specifically designed to improve sperm transport in the uterus and to optimize survival of viable spermatozoa in the mares' reproductive tract.
- The dosage is easily prepared using only a single bottle for the reinforcement breeding of each mare. After thawing the extender is used at semen temperature.
- Antibiotics are included within the extender; therefore there is no need to add any additional antibiotics.

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EQUIPRO<sup>®</sup> Reinforcer contains Amikacin and Ticarcillin. The extender is stored at -20°C and used within 24 hours of thawing and can be ordered with the REF.: 13570/0600.

# **Reinforcement breeding**

For equine breeding programs which require natural mount, like the Thoroughbred industry, the technique of reinforcement breeding can help to improve pregnancy success. Another term used for reinforcement breeding is "termed impregnation".

Reinforcement breeding involves obtaining a dismount semen sample from the penis of the stallion or from the mare's vagina immediately after natural mating, diluting this semen with an appropriate medium like EquiPro Reinforcer, and transferring the sample deeply into the uterus of the just mated mare with a Universal flexible pipette. It is reported that fertility is improved with this technique, probably because of an increased number of sperm reaching the uterus and being transported to the oviducts.

While stallion management can be accomplished by reviewing stallion breeding records and habits, reinforcement breeding is a technique to help ensure mares are successfully bred, especially when considering the variability of the natural covering habits of stallions.

## Poster Equine Reproduction. Order now!

Stallion and Mare Versions available. Size: 100 x 70 cm Language: English

Please order your quantities by sending an E-Mail to minitube@minitube.de, keyword: Poster Equine. Please don't forget to include your complete address. We will send out the posters immediately to your attention!

# **Computerized Semen Analysis with**

Sperm Vision<sup>®</sup> Therio is ideal for veterinary clinics, practitioners and semen labs. This system offers a highly efficient, flexible and sophisticated digital tool for evaluation of sperm.

Concentration, motility, morphology and viability (optionally) can all be accurately assessed, making Sperm Vision<sup>®</sup> Therio an essential tool for everything from donor evaluation to semen processing.

The user interface is straight forward, easy to use and can be customized through the preference menu to suit individual needs. Sperm Vision<sup>®</sup> Therio ensures evaluation integrity of both new and archived records through a password protected multi-level operational system. These features and many more make Sperm Vision<sup>®</sup> Therio ideal for the serious animal reproductive specialist.





Sperm Vision<sup>®</sup> Therio reports offer clear and concise sample analysis, featuring donor data, charts, graphs and video images. The user defined preferences allow for custom reports that may be delivered as hard copy or electronic files.

#### **Your benefits**

- Designed specifically for the needs of veterinarians, clinics and labs
- Advanced customizable record keeping capabilities for client, donor, referring veterinarian and associated data
- Accurate concentration, motility and morphology
  analysis
- Customizable reports, including photographs, charts, graphs and video images



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