The international AI news from Minitüb

Sperm Notes

ICAR Congress 2008 awards Simmet Prize for Assisted Reproduction

Minitüb’s CombiSystem: not only for semen!

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ICB – Minitüb’s International Center for Biotechnology in the US

The importance of acrosome integrity for mammal semen
ICAR Congress 2008 awards Simmet Prize for Assisted Reproduction

The highlight of this year’s ICAR was the first bestowal of the Simmet Prize for Assisted Reproduction. The Italian scientist Dr. Cesare Galli was awarded the Prize endowed with 50,000 EUR under the eyes of the assembled industry. More than 1100 participants had registered for the congress.

This prize is a memorial honoring the lifetime and legacy of Dr. Ludwig Simmet, who showed how an industry can be formed and created significantly through straightforwardness, integrity and staying power. Thirty-four years ago, he had laid the foundation for his exemplary career with the presentation of his first invention at the ICAR in Munich. Prof. Dr. Karl Fritz Weitze, a close friend of the family and long-time companion of founder of Minitüb, who died last year, looked back on his life in his introductory speech. Following the award ceremony, Ludwig O. Simmet, representing the family, thanked the members of the ICAR committee for the inspiration to honor Dr. Ludwig Simmet’s lifework in such a way.

The renowned committee had chosen Dr. Cesare Galli from a group of highly skilled candidates, who excel by innovative research, significant scientific progress in their field as well as by exemplary work within and outside science. A number of important papers have been published by Dr. Galli, among others constitutive work for the establishment of a standard protocol for the in vitro production of bovine embryos. The first successful cloning of a horse is to his credit also. An outstanding contribution for research and application of assisted reproduction was the foundation of LTR (Laboratorio di Tecnologie della Riproduzione) in 1992. His longtime membership in the AETE (Association Européenne de Transfer Embryonnaire), whose president he was for 4 years, also points out his efforts for technical progress and knowledge transfer from research to practice.

The Simmet Prize will be awarded every four years on the occasion of future ICAR meetings. The trust, founded by Dr. Ludwig Simmet’s 4 sons shall enable the ICAR to recognize an active research scientist for outstanding basic and applied research in the field of Assisted Reproduction of Animals, published during the previous five years.

Milestones in Dr. Cesare Galli’s vita:

1961 born in Erba, Italy
1981 - 1986 studies at University of Milan
1992 - present Director of LTR
1996 - 2000 President of AETE
2003 world’s first cloning of a horse
2004 - present professor at University of Bologna
Dr. Galli is married and has 3 children
Minitüb’s CombiSystem is well known to bull breeding stations worldwide: consisting of the filling and sealing machine MPPQuattro and the MiniJet printer Leibinger, it is a system solution that can handle the entire process - filling, sealing and printing of semen straws - in only one step. Just recently, a new application for this combination has been found: straws and CombiSystem are used in the biotechnology field to preserve microbiological cell cultures.

The client, a company specializing in microbiology cultures, uses normal 0.5 cc semen straws to package cultured bacteria with a culture medium containing freezing protectants. The straws are previously sterilized by gamma irradiation. After packaging and labeling with the CombiSystem, the straws are frozen and stored in liquid nitrogen.

The work with bacteria requires a high level of sterility. The CombiSystem is therefore placed in a so called Flowbox, where it is submitted to UV radiation for 2 hours every day. The CombiSystem is suitable for this treatment because of being relatively compact and closed. This helps to prevent any contamination coming from outside the system. Minitüb delivered this CombiSystem with special covers to protect parts of it which are not resistant enough to the UV light.

Another point which makes Minitüb’s CombiSystem especially suitable for the work with microbiologic cultures is the ease of cleaning and disinfection of all parts.

The operators in this specialized lab are very satisfied with the system and explicitly compliment the user friendliness as well as the reliability of the MiniJet Leibinger. The possibility of printing barcodes on each straw will be used in the future to simplify their handling.

Herewith a topic is picked up, whose development is being followed with great interest in the animal breeding industry. The trend definitely shows in one direction: the future of printing semen straws will include barcodes. Not only with the foundation of an ICAR task force, efforts are intensified to set up an international standard for coding straws to ensure documentation and traceability. Being a pioneer on implementation in this field, Minitüb will assist you with words and deeds if required.

CombiSystem, consisting of MPP Quattro and MiniJet REF.: 13018/0002
The Minitüb booth was extremely busy during the entire course of the International Congress on Animal Reproduction. 40m² of booth space were home to a large number of innovations that were presented by Minitüb to a great number of visitors. Here is a short overview:

**Gender identification of bovine embryos**

The brand new kit to identify the gender of bovine embryos created exceptional interest. SEX-Y™ contains a molecular probe known as Bov-Y that recognizes a specific site found only on the DNA of the Y chromosome. To identify the embryo’s sex, a biopsy is taken from the embryo. The extracted cells are then treated with the Bov-Y probe. If the embryo is a male, the Bov-Y probe attaches directly to the DNA of the Y chromosome and produces a fluorescent signal seen as a bright “dot” under a fluorescent microscope. If the embryo is female, there is no Y chromosome present, so there is no attachment of the Bov-Y probe and no fluorescent signal is observed. Thus, it is possible for the first time to determine the gender of an embryo in less than an hour without the complex and error-prone PCR technology.

**Automated progesterone test for effective bovine reproduction management**

With the eProCheck®, the first device worldwide was presented for analyzing fully automatically the Progesterone level in milk or blood serum of a cow, dog and soon of a mare also. Progesterone can be a very helpful support, especially for determination of the best insemination time and for early pregnancy detection. The test can be done by the farmer or the veterinarian right on the farm. The analysis system is based on ELISA.

**Processing bull semen with RFID**

The processing line for bull semen, starting with semen collection and ending with the labeled straw, has been continuously improved. From now on it is possible to read the electronic identification of the bull (earmark chip) wirelessly via WLAN-scanner. This battery-free reader is supplied with power by impulse. This impulse is simultaneously responsible for the data transfer to the laboratory software.

In future, the printing of semen straws with machine-readable barcodes will become more and more important. Printers from Minitüb have been able for many years to print barcodes on straws. Presenting the laser scanner with Bluetooth interface at the congress, Minitüb offers insemination technicians and farmers the option to read straws wirelessly via a barcode reader and then to import the data into the existing system.

**Insemination catheter with Poly Gel tip for sows**

Minitüb has developed a completely new generation of catheters for the artificial insemination of sows: ClearGlide™. The unique molecular structure of the poly-gel tip enables a superior lock of the catheter in the cervix. The flexible material of the tip adjusts to the cervix, seals it towards the back and prevents semen backflow. Thanks to the extraordinary gliding capacity and to the recessed shaft, insertion is effortless and well being of the animal is improved during breeding.

**Pheromone sprays**

With PheroBoar™ and PheroMate™ two new pheromone sprays were presented that are used as aids during AI of sows and semen production. PheroBoar™ is a classical boar spray for detecting estrus in sows and gilts. PheroMate™ assists with the training of boars and increases the vocalization and attentiveness of the animals.
**Minitüb: innovations presented at the ICAR in July 2008**

**MAVIC™ canine insemination catheter**

The new MAVIC™ catheter for artificial insemination of bitches is an advanced and technically mature product: the catheter was designed to mimic the natural breeding process. It stimulates uterine contractions, improving and facilitating sperm transfer to the oviducts. Simultaneously the unique balloon and injection system of MAVIC™ prevents unwanted back flow of semen. Thus it is ideal for the use of fresh or cooled semen. MAVIC™ is available in two sizes to accommodate a variety of breeds.

**Trans-cervical insemination of bitches**

New as well: the TCI endoscope for the trans-cervical insemination of bitches. Due to its length it can be applied for all dogs - no matter what size or weight. The ocular of the endoscope is angular, which eases handling during introduction and a working channel is provided for TCI insemination catheter. To operate safely, less training is necessary compared to TCI with other devices.

**Laser-technology in the field of Assisted Reproduction**

The OCTAX Laser System, which enables a safe and reliable microsurgery of the Zona Pellucida in many applications: assisted hatching, biopsy (also enucleation), laser-assisted intracytoplasmic sperm injection (ICSI) or in-vitro fertilization (IVF). The picture to the right exemplarily shows an electron microscopic shot of an oocyte of a mouse, whose Zona Pellucida was opened quickly and precisely with the OCTAX Laser. The visitors were able to convince themselves of the outstanding image quality and easy way of working with the OCTAX Laser system. Besides the laser system the high-resolution visualization of oocytes that is used for their evaluation was also shown.

**Vitrification of embryos**

New in the Minitüb product range for equine and bovine ET: The EquiPro Vit-Kit™ and BoviPro Vit-Kit™. A simple, fast and proven procedure for the cryopreservation of embryos. The vitrification process avoids crystallization of water molecules and thus causes less cell damage. No specialized freezing equipment is required.

**Deep intrauterine insemination of mares under visual control**

The new video-endoscope from Minitüb allows a semen dose to be deposited under visual control directly at the utero-tubal junction. A successful fertilization in the horse, especially when working with small amounts of semen, can be supported by the endoscopic method. Especially important: All parts of the endoscope which come into contact with the genital tract of the mare are exchangeable, enabling ideal hygiene of the process. Optionally, Minitüb offers a work station for the endoscope.

The EndoCenter contains a high resolution TFT-monitor, a light source and a pump for water/air-insufflation.
Where is the way leading for Assisted Reproduction in the future? The International Center for Biotechnology (ICB), founded by Minitube in 2004, tries to answer this essential and fascinating question. The area covers about 30 hectares, located in Wisconsin, USA, one hour of travel to the east of Milwaukee.

International accredited and established scientists work together with Minitub scientists to develop practical applications for animal and for human reproduction. Many products from the Minitub assortment like embryo transfer media, micro flush systems, and computer assisted analysis systems (e.g. SpermVision™) are the result of this work. Now these products are manufactured for commercial use.

Thus, the ICB is far more than only a research station: it is also a center for product development and quality control. Services for assisted reproduction like cloning and embryo transfer are offered as well. Furthermore, the center is a place where knowledge is communicated: apprenticeships, advanced training and training courses are offered as well. And, in cooperation with the research program, students of regional high schools as well as those from technical or veterinary universities are welcome to participate in courses and learning programs.

Connected to the ICB is a horse stud farm. The ICB offers facilities to keep horses, pigs, cattle, and dogs temporarily or permanently.

The importance of acrosome integrity for mammal semen

Today, quality of mammal semen can be determined more exactly than ever. Besides semen motility, semen morphology, and here especially acrosome integrity is one of the most important criteria to determine fertilization capability of an ejaculate. To understand this attribute correctly, the development of the spermatozoon from ejaculation to successful fertilization needs to be understood, as a cascade of processes is necessary until the semen cell is capable of fertilization and thus ready for entering the oocyte.

In the phase of the so-called capacitation taking place naturally in the genital tract of the female animal, the semen cell experiences a maturation that ends in a sperm cell being capable of fertilization. A series of biochemical changes result in the acrosomal membrane being capable of binding to the plasma membrane of the oocyte. This so-called acrosome reaction occurs when sperm cell and oocyte meet in the fallopian tube and bind to each other. There are two different processes that need to be distinguished: first the spermatozoa get into easy contact with the outer mantle of the ovum, the so-called zona pellucida (Z.P.). Next, the sperm cells dock to the Z.P (primary acrosome reaction). In the secondary acrosome reaction the outer membranes of the sperm and the oocyte merge. Enzymes, found in the acrosome, e.g. acrosin and hyaluronidase, are necessary for this process.

After penetration of the sperm cell, the zona pellucida changes and becomes impermeable for other semen cells. Thus, it is ensured that only one sperm can penetrate the oocyte.

For the procedure of this complicated and exactly coordinated process it is important that the sperm cell can fully capacitate and the acrosome is intact. Additionally, a certain quorum of fertile sperms per oocyte is necessary. It is assumed, that
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“helping sperms” with large numbers of lytic enzymes are necessary for a successful penetration of the Z.P.

Against this background the Minitüb International Center for Biotechnology (ICB, see own article) developed a method for acrosome analysis of boar, stallion and bull semen to indicate quickly and easily the number of damaged/infertile sperm cells. As a more exact quality measurement of the ejaculate is possible, the sperm numbers in semen portions from genetically valuable sires can be reduced to a minimum.

Besides the quality measurement of semen doses, their preservation is of great importance. It is the objective of any high quality semen extender to protect the acrosome membrane in its functionality during storage as it is exposed to free radicals, acid ions and maybe bacterial toxins. An ideal mixture of nutrients, buffers and ions can provide excellent protection. On the other hand, hen’s egg yolk used in some extenders, being rich of progesterone and its precursors (pregnenolone; Hartmann, 1998) can stimulate early acrosome reactions (Koehn, 1998). It is known that the contacting of the capacitating sperm cell with the Z.P. and hence the acrosome reaction proceeds under involvement of this hormone. The right choice of preservation medium finally saves a large number of sperm cells for insemination.

Rudolf Großfeld, PhD
Minitüb, grossfeld@minitube.de

References


Design of a sperm cell
Source: Dr. R. G. Saacke, Amer. J. Anat. 115: 143-184, 1964

www.minitube.de minitube@minitube.de
Impressions of ICAR 2008 in Budapest

!!! EuroTier in Hannover from 11.-14. November 2008 !!!

If we have aroused your interest, come and visit us at EuroTier in Hall 13, Stand E 57. All innovations presented in this issue are waiting there for you - and much more in the field of AI, ET and related techniques.

EuroTier takes place every two years in rotation with Agritechnica and is the leading exhibition for agricultural animal husbandry in Europe.
You can find more information at: www.eurotier.de