Freezing of Boar Semen

Freezing of boar semen is of great importance for the storage of genetic material in order to preserve and protect genetic variety. Furthermore, freezing facilitates the exchange of genetic material between geographically distanced breeding populations or **at times when trading from a biosecurity or epidemiologic point of view with fresh semen is problematic or not allowed.**

However, boar sperm are more sensitive to the processes of freezing than sperm of other species. The Minitube group provides a number of extender media, devices and procedures to reduce stress for boar sperm cells during the freezing process and to **ensure insemination success with frozen semen**.

minitube

Step 1

Collection/Centrifugation

- Using the gloved hand technique, collect the boar's sperm-rich ejaculate fraction (75 - 125 ml).
- Measure the volume.
- Determine motility and sperm concentration, preferably with **AndroVision®**.
  Minimum required motility: 75%
- Determine percentage of normal cells.
  Minimum required: 90%
- Calculate **TSE** which is the **T**otal number of **S**perm **c**ells in the Ejaculate multiplying the volume of ejaculate by sperm cells/ml in the ejaculate.
- Dilute the ejaculate 1:1.5 by volume with **Androstar® Premium** at approx. +30°C.
  Semen and extender should have the same temperature during dilution.
- Cool and maintain at +17°C over night or process immediately after the semen has been cooled to +17°C. If the semen is stored overnight a higher dilution of 1+5 is recommended.
- Centrifuge at +17°C, 800 g for 12.5 to 20 minutes, preferably in 50 ml to 500 ml conical centrifuge tubes.
- Aspirate the supernatant and determine its volume and sperm concentration.
- Re-suspend with **CryoGuard** Part A cooling extender to a total volume of approx. 25 to 30 ml. Semen and extender should have the same temperature during dilution.

Cooling Extender and Egg Yolk Preparation

Please make sure to proceed exactly in the described order when preparing the stock solution.

Preparation of 100 ml CryoGuard Part A cooling extender:

- Warm up 80 ml bi-distilled water to +32°C, up to +38°C.
- Dissolve 1 package of CryoGuard Part A in the water.
- Allow the pH value to stabilize for 20 minutes, until the powder is completely dissolved.
- Carefully stir this solution into 20 ml of fresh egg yolk.

Make sure you add the CryoGuard Part A solution to the egg yolk and NOT vice versa. Let the solution cool to +17°C.

This is the cooling extender.

Freezing Extender Preparation

- Add 50 ml of bi-distilled water to CryoGuard Part B.
- Add this solution to 20 ml of egg yolk (add Part B medium to egg yolk, not vice versa).
- Homogenize the freezing extender completely i.e by applying a magnetic stirrer.
- Let the freezing extender cool down to +5°C.

This is the freezing extender.

AndroVision®

This highly precise automated system for computerised semen analysis stands out for the integration of the classic CASA analysis with more advanced assays for sperm functionality.

AndroVision® software
with PC and accessories REF.: 12500/0000

Laboratory scales

Precision scale for the determination of the ejaculate volume or for weighing out extender, weighing range: 6000 g, resolution 1 g, weighing pan 150 x 170 mm

REF. : 14273/0440

Precision scale for accurate weighing of antibiotics or extender powder, weighing range: 2000 g, resolution 0.1 g, weighing pan 130 x 130 mm

REF. : 14295/0444

Photometer SDM 1

The SDM 1 is a compact, highly accurate photometer developed for measuring sperm concentration.

Photometer SDM 1 REF. : 12300/0100

Androstar® Premium

Androstar® Premium long-term extender for boar semen is based on an innovative combination of protective molecules.

Androstar® Premium
1 litre REF. : 13533/1001
5 litres REF. : 13533/1005
10 litres REF. : 13533/1010
100 litres REF. : 13533/1100

Refrigerated centrifuge

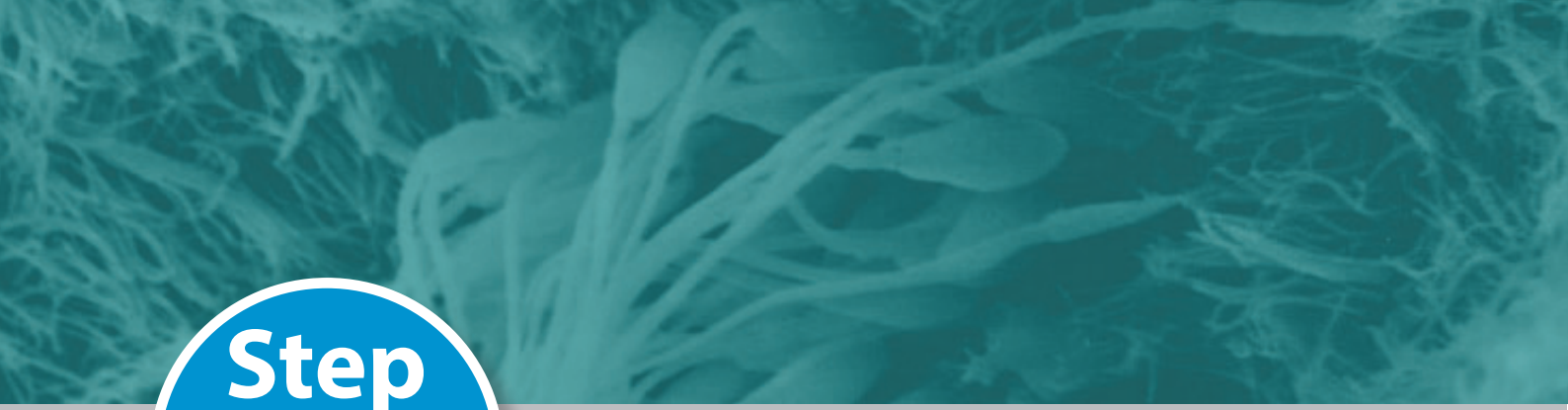
With swing out rotor, 4 buckets and 4 x 600 ml polypropylene bottles with caps.

Refrigerated centrifuge REF. : 14602/0400

Low density vacuum pump, 30-60 Hg, with manometer and valve
230 V/50 Hz REF. : 13133/0267

Condensor bottle, 500ml REF. : 5013020/0095
Silicone tubing ø 5 x 8 mm REF. : 5013101/0100

Step 1



Step 2

Semen Extending/Cooling

Add **CryoGuard** Part A cooling extender and cool

- Calculate **TSS** which is the **T**otal number of **S**perm cells in the **S**upernatant multiplying the volume of the supernatant by sperm cells/ml in the supernatant.
- Calculate **TSC** which is the new **T**otal number of **S**perm cells re-suspended in the **C**ooling extender by subtracting **TSS** from **TSE**. Add **CryoGuard** Part A cooling extender to the re-suspended semen pellet until reaching 50% of the final volume. Semen and extender should have the same temperature during dilution.

Proceed as follows:
Calculation of **CryoGuard** Part A cooling extender volume:

Vol. (ml) ejaculate x concentration (10^9 sperm/ml) = **TSE**
Vol. (ml) supernatant x concentration (10^9 sperm/ml) = **TSS**
TSC = TSE – TSS

In order to obtain $4x10^9$ sperm cells/dose (8 straws x 0.5 ml = 4 ml), 1 ml must contain $1x10^9$ sperm cells:

TSC (billion sperm cells) = **Final volume** (ml)

Example:

Volume of the ejaculate = 90 ml
Semen concentration = 900 million/ml
TSE: 81 billion sperm cells (81×10^9)
Volume of the aspirated supernatant = 210 ml
Semen concentration in the supernatant = 28.57 million/ml
TSS: 6 billion sperm cells

TSC = 81 – 6 = 75 billion sperm cells

CryoGuard Part A cooling extender needed:

Example:

TSC = 75 billion cells
Final Volume = 75 ml
Total volume of semen pellet with cooling extender: 37.5 ml
Total volume of freezing extender: 37.5 ml

The final sperm cell concentration will be 1 billion sperm cells/ml

Freezing extenders for boar semen

CryoGuard (part A)
Powder, makes 100 ml cooling extender.

REF. : 13514/2100

CryoGuard (part B)
Liquid, contains Glycerol, for preparation of 100 ml Part B freezing extender.

REF. : 13514/2110

CryoGuard Thaw,
thawing extender for frozen boar semen,
powder for 1000 ml extender.

REF. : 13514/2200

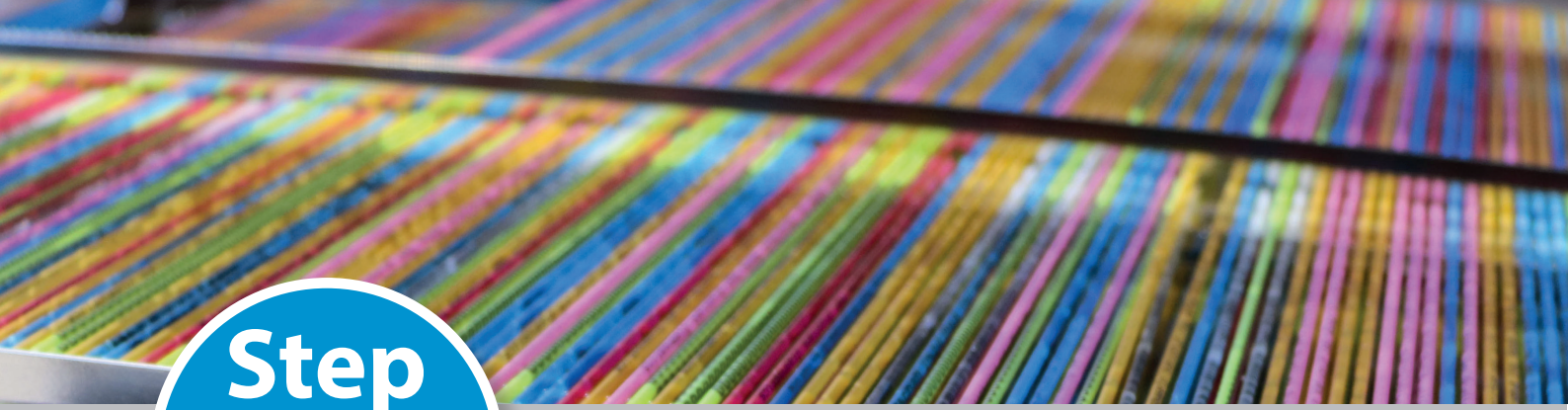
Cooling cabinet

Temperature preset to 5°C.

Cooling cabinet REF.: 14335/3000

Step 2

- Actual weight of re-suspended pellet may be determined by weighting empty centrifuge tube or bottle before adding the semen and re-weighting container after aspirating the supernatant.
- Finish adding cooling extender to the re-suspended pellet when reaching 50% of the final volume.
- Place semen in **+5°C cooling cabinet** or cold room and slowly cool for 1.5 hours.
- Monitor temperature. When semen temperature reaches +5°C, proceed with step 3.



Step 3

Add Extender and Fill Straws

- Complete dilution by slowly adding the calculated ml of **CryoGuard Part B f**reezing extender at +5°C. Semen and extender should have the same temperature during dilution. Proceed immediately with straw filling and sealing.
- Use pre-printed straws (preferably **MultiCoder** or **EasyCoder** technique).
- Fill 0.5 ml straws with the **SFS semiautomatic filling and sealing system**. An **MPP Uno** can serve as an alternative for filling straws.

TSC x 2 = approximate number of required straws
Example: 75 x 2 = 150 straws

- Fill and seal straws in a +5°C cold room or cooling cabinet.
- Shake air bubble to centre of straws by shaking the magazine with filled and sealed straws.

Semen straws

Color	Medium Straw 0.5 ml	EcoStraw 0.5 ml
Transparent	13408/0010	13408/3010
Red - transparent	13408/0044	13408/3044
Green - transparent	13408/0054	13408/3054
Blue - transparent	13408/0064	13408/3064
Grey - transparent	13408/0074	13408/3074
Yellow - transparent	13408/0094	13408/3094
Pink	13408/0100	13408/3100
Turquoise	13408/0130	13408/3130
Orange	13408/0140	13408/3140
Orange - transparent	13408/0144	13408/3144
Pistachio green	13408/0200	13408/3200
Pistachio green - transparent	13408/0204	13408/3204
White	13408/0210	13408/3210

Straws can also be delivered pre-printed.
Please ask for the Minitube printing service!

Macrotubes

2.5 ml, length: 140 mm — REF. : 13441/0133
4 ml, length: 240 mm — REF. : 13441/0240
5 ml, length: 280 mm — REF. : 13441/0280

Sealing balls for macrotubes:
Metal or glass, available in many different colors.

Semiautomatic system SFS 133

Semiautomatic filling and sealing machine SFS 133,
with vacuum bottle, filling and suction head
for 0.5 ml straws — REF. : 13133/0133

Low density vacuum pump, 30-60 Hg, with manometer and valve
230 V/50 Hz — REF. : 13133/0267
115 V/50 Hz — REF. : 13133/0268

Cartridge loading device with loading block and
10 cartridges for 36 straws 0.5 ml — REF. : 13133/0005

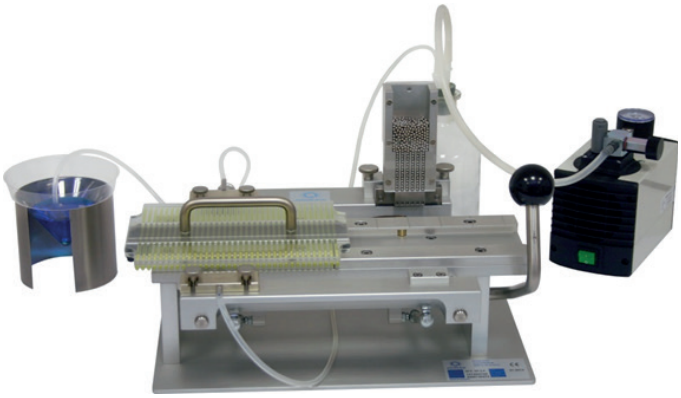
Filling head with 6 nozzles — REF. : 13133/1000

Suction head with 6 nozzles — REF. : 13133/2000

Silicone tubing for SFS transparent, 3 x 1 mm — REF. : 13133/3550

Washer for all filling and suction nozzles,
5000/bag — REF. : 13021/3001

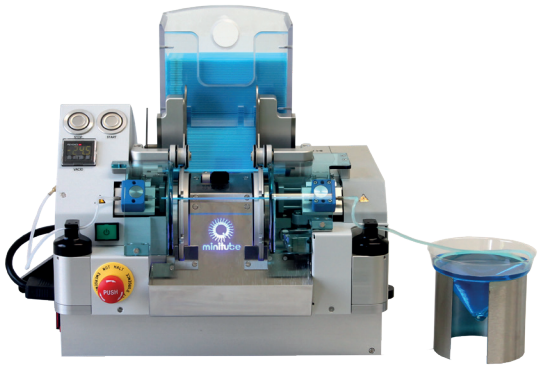
Metal sealing ball for 0.5 ml straw — REF. : 13400/9900



MPP Uno

Automatic filling and sealing machine for straws.

MPP Uno, complete — REF. : 13017/0000

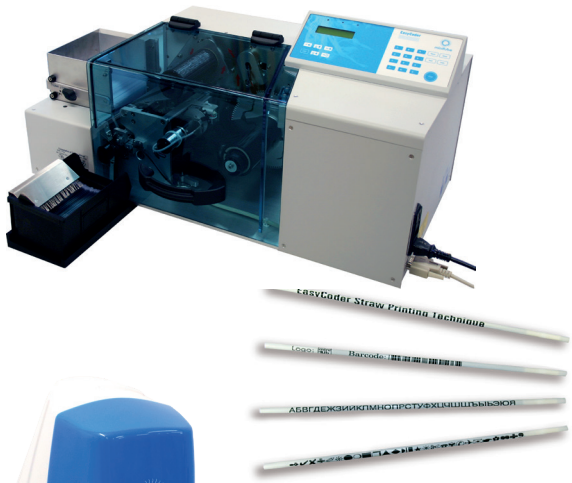


EasyCoder

Thermo transfer printer for semen straws with automatic straw feeder.

EasyCoder — REF. : 13038/0000

Ink ribbon for straw printer EasyCoder,
roll with 200 m, black — REF. : 13038/0010

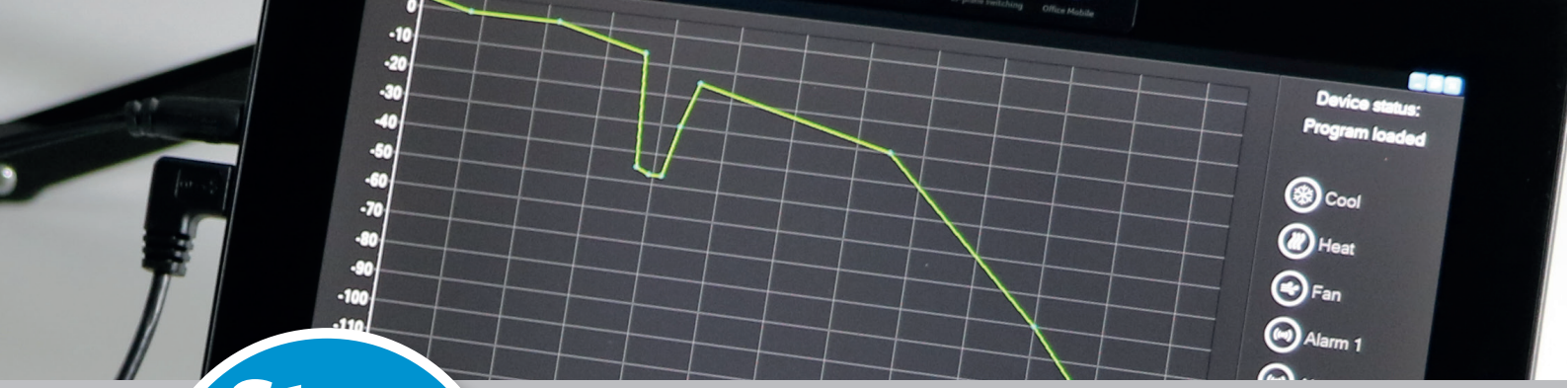


MultiCoder

Professionalize your lab process with quick and easy machine readable labeling of samples.

MultiCoder printer — REF. : 13145/0000

Step 3



Step 4

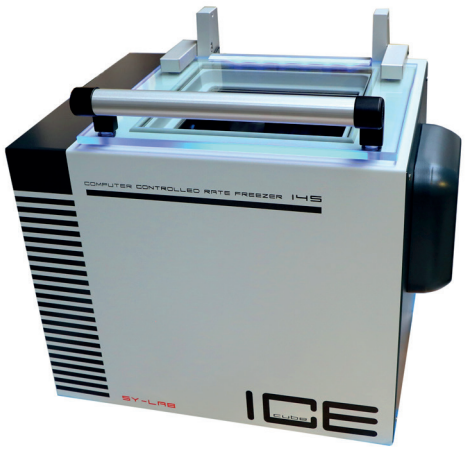
Freeze Semen Straws

- Freeze in a programmable freezing chamber (**IceCube or TurboFreezer M)** at a decreasing temperature rate of 30°C/minute.
- Remove straws from **IceCube/TurboFreezer** and immediately plunge into liquid nitrogen (styrofoam box).
- Submerge canes with attached goblets in liquid nitrogen.
- Fill goblets with straws using forceps or use cassettes as an alternative to goblets.
- Quickly transfer to bulk liquid nitrogen storage.

IceCube

Computer controlled freezer with user-friendly data input and automatic recording of the freezing curve.

IceCube 14S,
without monitor REF. : 16821/1000



TurboFreezer M

Minitube's TurboFreezer provides directional, continous nitrogen gas flow throughout the whole chamber and therefore uniquely uniform freezing patterns.

TurboFreezer M REF. : 16810/0000

Step 4

Storage systems for straws

Goblet
13 mm, white REF. : 16913/0133

Goblet cane
for 2 x 13 mm goblets REF. : 16965/6013

Short triangular cassette
for 10 straws 0.5 ml, white REF. : 16981/01241

Long triangular cassette
for 20 straws 0.5 ml, white REF. : 16981/01391

Liquid nitrogen container REF. : 16503/2056

We offer a variety of liquid nitrogen containers, please ask for further information!

Step 5

Semen Thawing and AI Dose Preparation

- Remove 2–3 straws from **liquid nitrogen storage container** and immediately transfer to the thaw unit at +38°C.
- Thaw semen straws for 20 seconds and remove.
- Wipe straws with a clean paper towel.
- Shake air bubble to the sealing ball end and cut straws at ball end, holding cut end up.
- Empty straws into pre-warmed semen tube (e.g. **QuickTip Flexitube®**) at +26°C. Invert straws over the tube and cut the cotton-plugged end to release the semen.
- Repeat this step to achieve the desired number of viable cells per dose (~4 billion or 8 straws).
- Slowly add 60 ml of pre-warmed thawing solution (**CryoGuard Thaw** should be used as thawing solution).
- For motility evaluation, transfer a semen sample to a sample tube and warm at +37°C for 15 to 20 minutes. Evaluate on pre-warmed slide under a phase contrast microscope with warming plate, subjectively or by using **AndroVision®**.
- Seal the semen tube with a **manual sealer** or **tube clamp.**

Thawing device MT

- Electronic temperature control
- Central thawing chamber with lift
- Accuracy ±0.2°C
- Power supply: 12 V DC (car battery)

MT 30/54: set to **+38°C** REF. : 17044/0038

Semen Tubes

Minitube tubes are available in different colors and sizes:

QuickTip Flexitube®, 95 ml, transparent REF. : 13452/03961

Tube sealer

3-tube manual sealer, 230 V REF. : 13225/0003
Tube magazine, 6 tubes REF. : 13204/9006

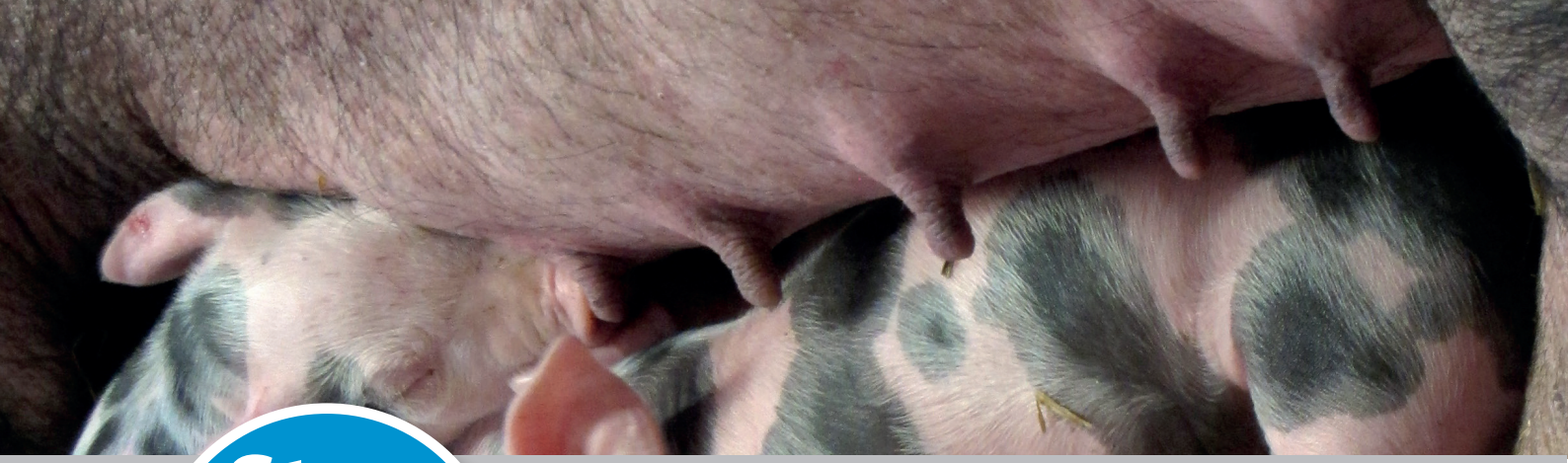
Tube clamp
to seal e.g. tubes of test samples REF. : 13452/0005

Boar semen bottles

Boar semen bottles, 500/box, 100 ml REF. : 13450/0100

Caps are available in different colors.

Step 5



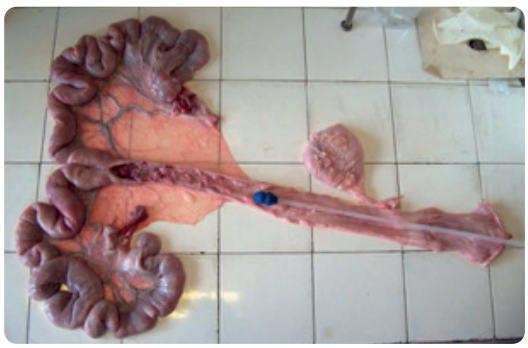
Step 6

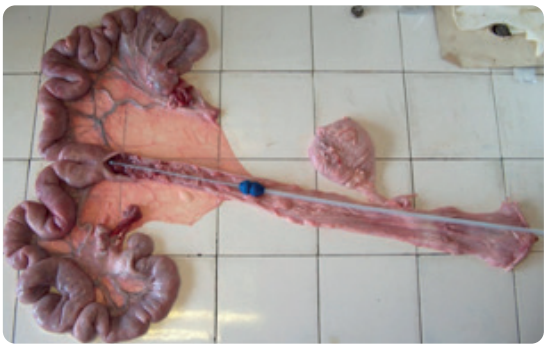
Inseminate Sows with Semen Dose and intrauterine Catheter

The best fertility results with frozen/thawed boar semen are obtained after intrauterine insemination with the Minitube **PC Blue** (Ref. 17112/2000), **PC Clear** (Ref. 17112/3000) or **DeepBlue catheter** (Ref. 17113/0100).

Tips and tricks

- The presence of a boar during PCAI insemination is not recommended. The stimulation of the sow by the boar causes contractions of the uterus which complicates the introduction of the PC-cannula. Therefore, it is better to do the heat detection separately and not at the same time of the insemination.
- Gilts can mostly be inseminated after the third oestrus with PC-AI. The reproductive tract and thus the cervix of younger animals are not yet sufficiently developed.
- The boar may be driven in the feed alley in front of the sows after the AI. The stimulation by the boar improves the sperm transport in the uterus of the sow.
- A good possibility for training PCAI is a uterus from the slaughter house. The procedure of this kind of insemination is easier to learn if one can feel the cervix passage and also see it.





For more information please have a look at our Leaflet: Post-cervical insemination in sows.

PCAI catheter

Your benefits

- Ideal for the post-cervical insemination of sows: all semen cells reach the uterus and can easier be transported into the fallopian tube by uterine contractions
- The fertility of the inseminated sow can be improved, or can be maintained with a reduced amount of sperm cells
- The Foamtip or ClearGlide® head of the outer catheter is already lubricated; the whole catheter is individually packed in the hygienic SafeBlue sheath
- Inner catheter with very small diameter: nearly no semen remains in the catheter
- Inner catheter optimized in material design and flexibility to glide along the cervix tissue; no blocking and kinking in cervix cushions
- SafeBlue concept garantuees high hygiene status

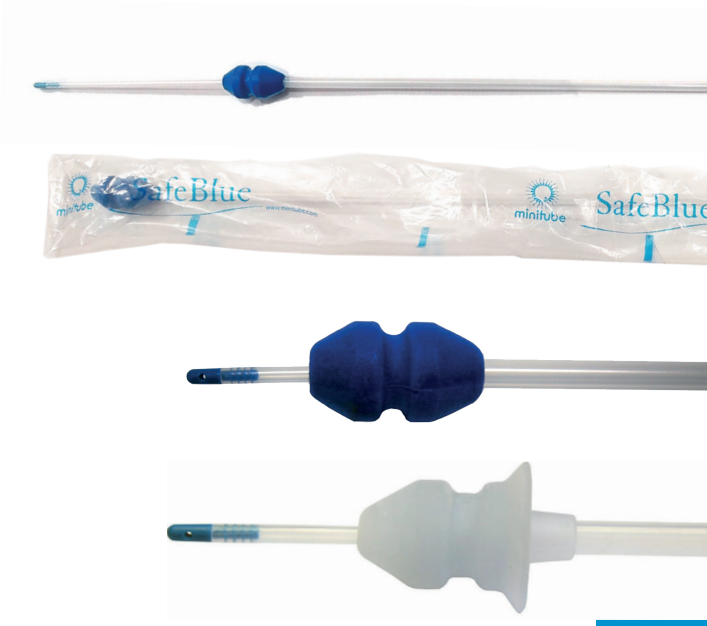
PC cannula for post-cervical insemination, 5/bag REF. : 17112/1010

PC Blue, SafeBlue Foamtip® with PC cannula, individually packaged, sterilized, 25/bag REF. : 17112/2000

PC Blue, SafeBlue Foamtip® with PC cannula and stopper, individually packaged, sterilized, 25/bag REF. : 17112/2002

PC Clear, SafeBlue ClearGlide® with PC cannula, individually packaged, sterilized, 25/bag REF. : 17112/3000

PC Gilt, SafeBlue SoftGilt with PC cannula, for postcervical insemination of gilts, 25/bag REF. : 17112/4000



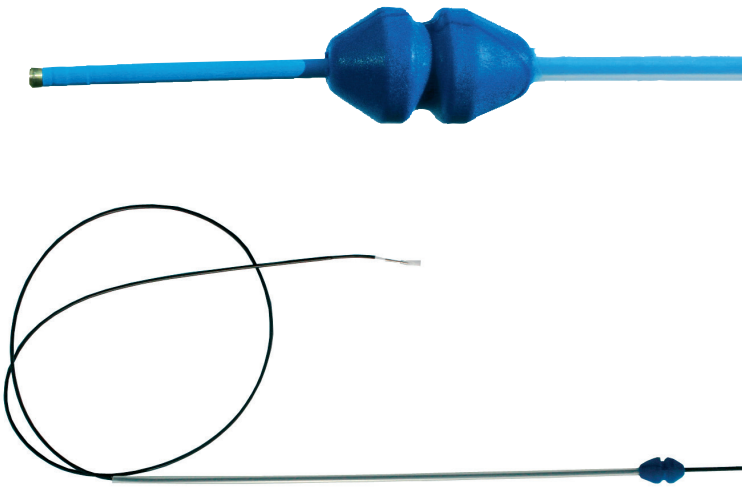
Step 6

DeepBlue AI catheter

Your benefits

- For deep intrauterine insemination of special semen doses: ideal for very low numbers of sperm, i.e. in frozen-thawed or sexed semen
- Flexible and at the same time firm and soft inner catheter, with a length of more than 1.5 m, can be inserted over the complete distance of an uterus horn up to its tip
- A small metal tip facilitates the entry into the cervix
- Patented (University of Murcia, Spain)

DeepBlue AI catheter, individually packaged, sterilised REF. : 17113/0100



Make your own Calculation

Calculation

	x		=	_____
Ejaculate volume (ml/g)		Sperm/ml in Ejaculate		Total number of sperm cells in ejaculate (TSE)
	x		=	_____
Supernatant (ml)		Sperm cells/ml in supernatant		Total number of sperm cells in supernatant (TSS)
_____	-	_____	=	_____
Total sperm number in ejaculate (TSE)		Total sperm cells in supernatant (TSS)		Total sperm cells in cooling extender (TSC)
_____	x	1ml	=	_____
Total number of sperm cells in cooling extender (TSC)				ml Final volume
_____	: 2		=	_____
Final volume				ml Cooling extender including re-suspended sperm cells
_____	: 2		=	_____
Final volume				ml Freezing extender
_____	x 2		=	_____
Final volume				approx. number of needed 0.5 ml straws
	: 8		=	_____
Number of straws				Number of semen doses with 4 billion sperm cells each

means: Put in measured/counted values.

Minitüb is an ISO 9001:2015 certified company.

04/2024

minitube

www.minitube.com