

Equine Product Manual

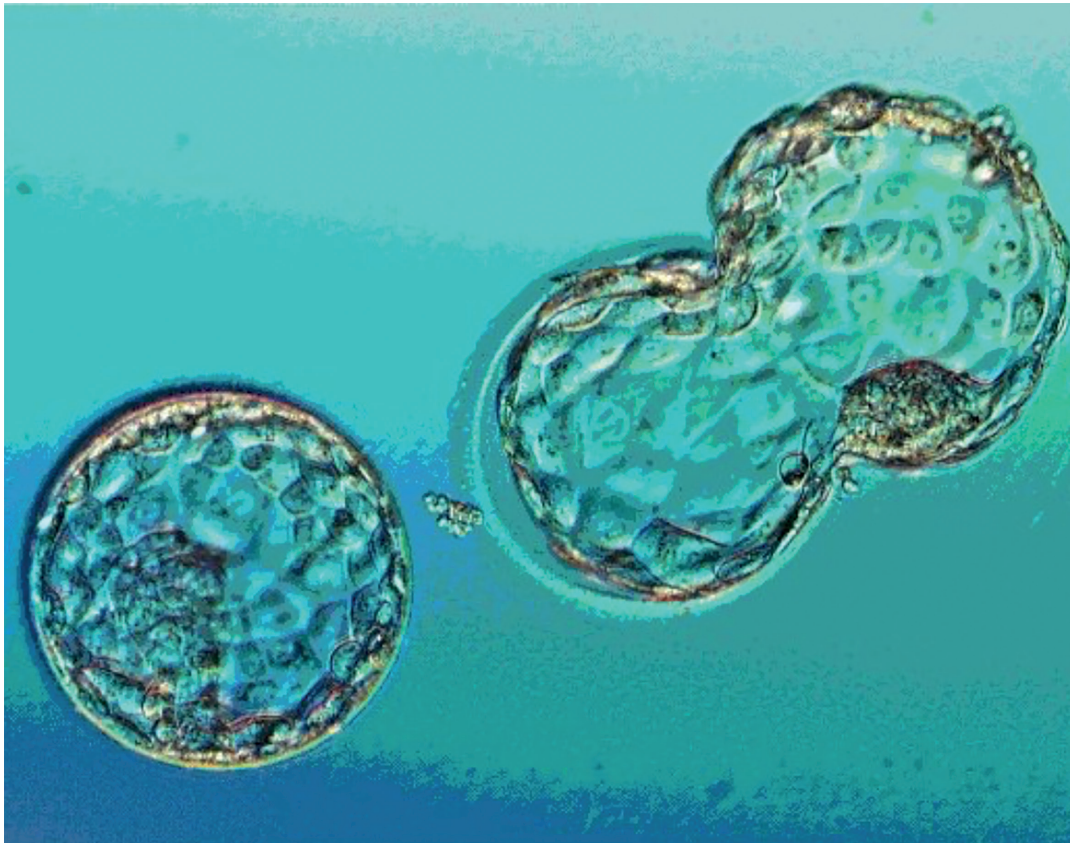


Nidation

1. The building of a nidus, a nest, as with birds.
2. Implantation of the fertilized ovum (zygote) and the building of a nest in the endometrium, the placenta.

Conception

1. The union of male and female gametes, the sperm and egg.
2. An impression or idea.



Contents

Introduction • Quality	4
Shelf life • Packaging	5
Products • Ordering information.....	6
Products	7-9
EquiPure – density centrifugation	10-13
Extenders	14-15
Freezing extender	16
Red Cushion.....	17
ICSI.....	18-19
Embryo transport media.....	20
Vitality and morphology assessment.....	21
References.....	22
Contacts.....	23

Introduction

Nidacon International AB manufactures and sells Medical Devices mainly for Assisted Reproduction Technologies (ART), with IVF, ICSI and insemination (IUI) solutions. The company was founded in 1996 by Assoc. Prof. Paul V. Holmes MSc, PhD, DrMedSc, an embryologist and endocrinologist from the Dept. of Obstetrics and Gynaecology at Sahlgrenska University Hospital in Gothenburg, Sweden.

Nidacon considers many different factors when designing its products. We hope that the attention to detail has helped to create products which will lead to better results. We aim to work in close relation with our customers; they are the cornerstones of our research department.

We take pride in the development of our products and make sure we respond to the needs of our customers and research colleagues. All our products are developed in close cooperation with professionals in the different fields.

At Nidacon, we take Corporate Social Responsibility seriously

We vow to do our best to help create and maintain a sustainable world, not just by adhering to the strictest environmental policies with regard to our products and production, but by taking responsibility for our impact on society as a whole. We will always strive to contribute in the best way to improve the world around us, both at home and abroad. After all, what point is there in helping to create new life if our world is not a sustainable place to live in?

Recycling

At Nidacon, we think a lot about our legacy. That's why we take responsibility for our impact on our world and work actively to ensure that our legacy is a positive one. The first step in this process is a more conscious use



of our finite resources. Having a well-functioning system for waste recycling is essential to maintaining healthy ecosystems and environments.

Carbon offsets

A Carbon Offset is a way to compensate for carbon emissions by funding an equivalent carbon dioxide saving elsewhere. Nidacon transport products all over the world, and while we use the most practical and sustainable forms of transport available, we have compensated for the carbon emissions caused by these transports by supporting carbon emissions reduction projects, helping to mitigate our environmental impact.

Biodiversity efforts

Unfortunately, biodiversity is on the decline. One example is the reduction in the number of honeybees and other pollinators, which now are becoming endangered species. This is especially dangerous since bees play a critical role in our ecosystem, with the majority of all plants and about one third of the world's crops, requiring cross-pollination to reproduce and thrive. For this reason, part of Nidacon's sustainability efforts is focused on helping to prevent further reduction of the honeybee population, thereby increasing in biodiversity and pollination in our local ecosystem.

Supporting street children of Nepal

Nidacon's entire business is helping couples fulfil their dreams of having a family. For this reason, we feel that we have an extra responsibility for the children in the world. We put a great deal of effort into selecting the organizations/projects we support and are pleased to have over the years been working with the "We Care" organization in this regard. The organization has three ongoing projects focused on helping "street children" in Nepal, focusing on improving the lives of these at-risk children and hopefully providing a better future.

Quality

Nidacon is certified according to SS-EN ISO 13485 (implemented 2003-08-15). The management system secures continued development of the organisation.

Nidacon intends to always maintain the high quality of its products and, in order to achieve this, all batches are tested at Nidacon before they are cleared for the market. A batch is only released for sale if it meets specific criteria.

Each batch is accompanied by a quality assurance certificate which records the results of the tests. Using this rigorous quality control system, we ensure that each batch meets the correct standards. Consequently the customers are secure in the knowledge that our products are reliable and will provide good results when used correctly.

Shelf life

EquiPure is stable for two years at room temperature. After opening, the products should be stored at 2 to 8°C when not in use.

The extenders are stable for two years in powder form. After reconstitution in water they can be stored in refrigerator for 3 days. It is also possible to freeze reconstituted

extender and store for up to a year. BotuCrio should be used within 3 days after thaw. It is possible to re-freeze, but there is then a risk of a build-up of fat from the egg yolk which is seen as black debris in the microscope. This is however harmless to the spermatozoa.

Packaging

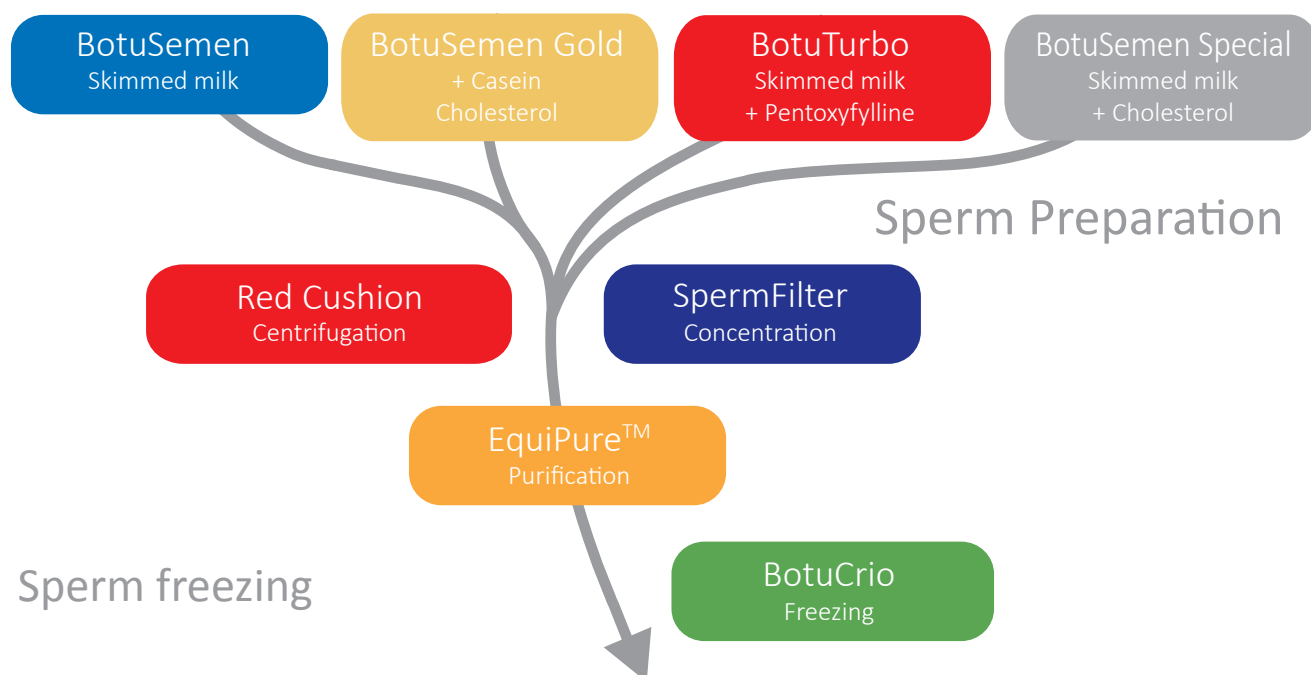
The packaging for Nidacon's products has received the same care and attention to detail as the

design of the products. All packaging are MEA-tested to ensure lack of toxicity.



Nidacon Equine Product Range

Semen extenders



Ordering information

Description	Volumen	Art. no.
EquiPure	EPB-100	100 mL
BotuSemen	BTS-100	100 mL
BotuSemen with water	BTSW-100	100 mL
BotuSemen Special	BTSS-100	100 mL
BotuSemen Special with water	BTSSW-100	100 mL
BotuSemen Gold	BTSG-100	100 mL
BotuSemen Gold with water	BTSGW-100	100 mL
BotuTurbo	BTT-100	100 mL
BotuTurbo with water	BTTW-100	100 mL

Description	Volumen	Art. no.
BotuCrio	BTC-025	25 mL
SpermFilter	BTF-001	1 pcs
RedCushion	RC-100	100 mL
SpermVitalStain	SVS-010	2x10 mL
NidOil	NO-100	100 mL
NidOil	NO-400K	4x100 mL
BotuEmbryo	BE-008	8 mL
SpermCatch	SC-100	6 × 100 µL
ProlInsert	PI15-5	5 pcs



EquiPure™

Ready-to-use density gradient solution, optimized for the preparation of density gradients for the separation and purification of equine sperm.

Silane coated, silica colloid in an isotonic solution.

Shelf-life: Two years at room temperature.



BotuSemen

Extender based on skimmed milk to transport cooled semen, to preserve at 5 or 15°C and for centrifugation pre-freeze. Contains Penicillin K 1 g/l and Gentamicine 1 g/l.

Suitable for most stallions.

Shelf-life: Two years in powder form, three days after reconstitution in water at 2-8°C, one year frozen after re-constitution in water.



BotuSemen Special

Extender based on skimmed milk with the addition of cholesterol.

BotuSemen Special is highly recommended for pre-freezing centrifugation and is suitable for spermatozoa with sensitivity to cooled transport.

Contains Penicillin K 1 g/l and Gentamicine 1 g/l.

Shelf-life: Two years in powder form, three days after reconstitution in water at 2-8°C, one year frozen after re-constitution in water.



BotuTurbo

Extender based on skimmed milk with addition of Pentoxifylline.

Ideal for fresh insemination or cooled semen transport at 5°C.

Suitable for stallions with poor sperm motility.

Contains Penicillin K 1 g/l and Gentamicine 1 g/l.

Shelf-life: Two years in powder form, three days after reconstitution in water at 2-8°C, one year frozen after re-constitution in water.

Products



BotuSemen Gold

Extender with casein and cholesterol. Suitable for all stallions, especially for those with suspected premature sperm capacitation, and with sensitivity to cooled transport.

Contains Penicillin K 1 g/l and Gentamicine 1 g/l.

Shelf-life: Two years in powder form, stored at 2-8°C. Three days after reconstitution in water at 2-8°C, one year frozen after re-constitution in water.



BotuCrio

Freezing extender with egg-yolk. BotuCrio contains a low concentration of glycerol, which in combination with one of several amides, results in better sperm integrity, sperm viability, better post-thaw total and progressive motility, as well as sperm velocity, compared to other commercially available freezing extenders.

Contains Gentamicine 0.5 g/l.

Shelf-life: 1 year frozen. Three days at 2-8°C after thaw.



RedCushion

Cushion fluid of high density used for semen centrifugation. It reduces damage from centrifugation through sperm damping over the RedCushion on the bottom of the tube. Because it has a reddish coloration, it allows for easy visualization of the sperm layer and its removal.

Shelf-life: 2 years at 2-8°C.



SpermFilter

Hydrophilic synthetic membrane which efficiently concentrates stallion sperm.

Shelf-life: Re-usable, stored at room temperature.



SpermCatch™

For slowing down sperm prior to ICSI using hyaluronic acid. To avoid ICSI injection of PVP, it contains only natural products for increasing the viscosity.

Shelf-life: 1 year at room temperature.



NidOil™

Sterile, light paraffin oil for use as an overlay during gamete, zygote and pre-embryo culture in the incubator, or during manipulations outside the incubator. No additives.

Shelf-life: 2 years at room temperature.



BotuEmbryo

BotuEmbryo is a media for maintenance and transport of embryos. Its packaging does not require the use of syringes and needles. It has an innovative formulation based on scientific studies carried out by national and international researchers.

Shipped at ambient temperature (up to 3 days).

Shelf-life: 1 year at 2-8°C.



Sperm VitalStain™

One step staining technique for assessment of equine sperm vitality and morphology, a basic tool for semen analysis.

Shelf-life: 2 years at 10-40°C.

EquiPure – density centrifugation

Background

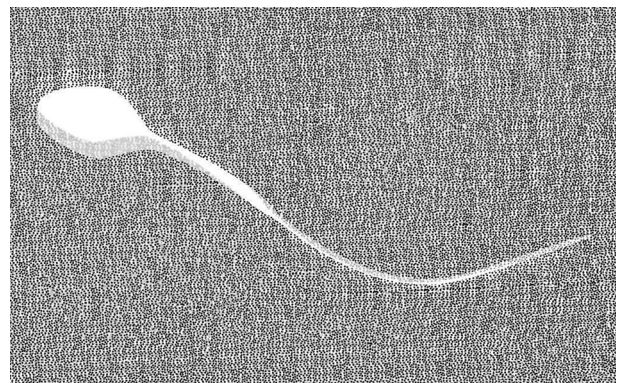
EquiPure is a product that helps increase fertilization and pregnancy rates by removing harmful agents from semen samples. Semen typically contains various cell types, including normal motile sperm, juvenile sperm, senescent sperm (which cannot fertilize), and sperm with DNA breaks. Additionally, semen contains epithelial cells,

immune cells, and cell debris (detritus), as well as bacteria and viruses. Biological substances such as sperm decapacitating factors and reactive oxygen species (ROS) are also present and can negatively affect fertilization. EquiPure works by removing all of these detrimental agents from the semen sample.



What is EquiPure and how does it work?

EquiPure is a substance that contains colloidal silica which has undergone the process of silanization. Colloids are particles that are small enough to not be affected by gravity ($\leq 1 \mu\text{m}$), but large enough ($> 1 \text{ nm}$) to not be considered a true solution. This gives EquiPure its unique functional characteristics. While silica occurs naturally in crystalline form and is abundant in the earth's crust, the silica in EquiPure is synthetically manufactured to meet specific requirements and specifications. This synthetic colloidal silica contains amorphous silicon dioxide (SiO_2) and has smooth, spherical particles that are dispersed in a fluid referred to as silica sol, which is essentially water. The dispersion is stable, meaning the particles do not settle or clump together at a significant rate. The solution is then adjusted with various salts to achieve the proper physiological composition and osmolality for equine spermatozoa. The pH and osmotic values are set to lie somewhere between those found in semen.



EquiPure™

Important:

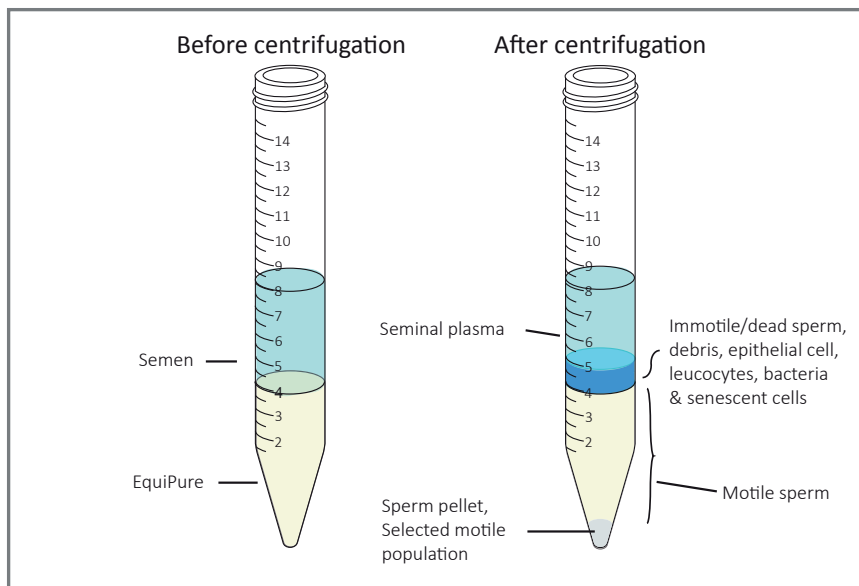
- The procedures described below should only be performed in centrifuges with swing-out rotor. Centrifuges with fixed angle rotor should not be used.
- EquiPure does not contain antibiotics, use aseptic procedures.
- The protocol states g-force and not RPM. It is important to have the correct g-force and we advise you to use the equation to make sure it's the

recommended g-force for the centrifugation. Different sizes of centrifuges requires different RPM to achieve the same g-force.

To achieve the correct g force:

$Rpm = \sqrt{\left[\frac{g}{(1.118 \times r)} \right]} \times 10^3$ r = rotational radius, the distance (mm) from the centre of the rotor to the bottom of a centrifuge tube in the bucket when raised to horizontal position.

A calculator can also be found on this QR-code.



General care and use

- All solutions should be brought up to room temperature before use to avoid the temperature fluctuations which are detrimental to sperm survival.
- Open and reseal bottles in a laminar air-flow bench (if possible) using sterile techniques to avoid contamination.
- Store all opened bottles at 2-8°C after re-sealing.
- Under these conditions the same shelf-life applies even after opening.

Tips

- When retrieving the pellet after the gradient centrifugation, care must be taken to avoid contaminating the pellet with components of the ejaculate. Therefore we recommend using a new pipette after removing most of the gradient to avoid contamination, for example, by bacteria.

EquiPure – density centrifugation

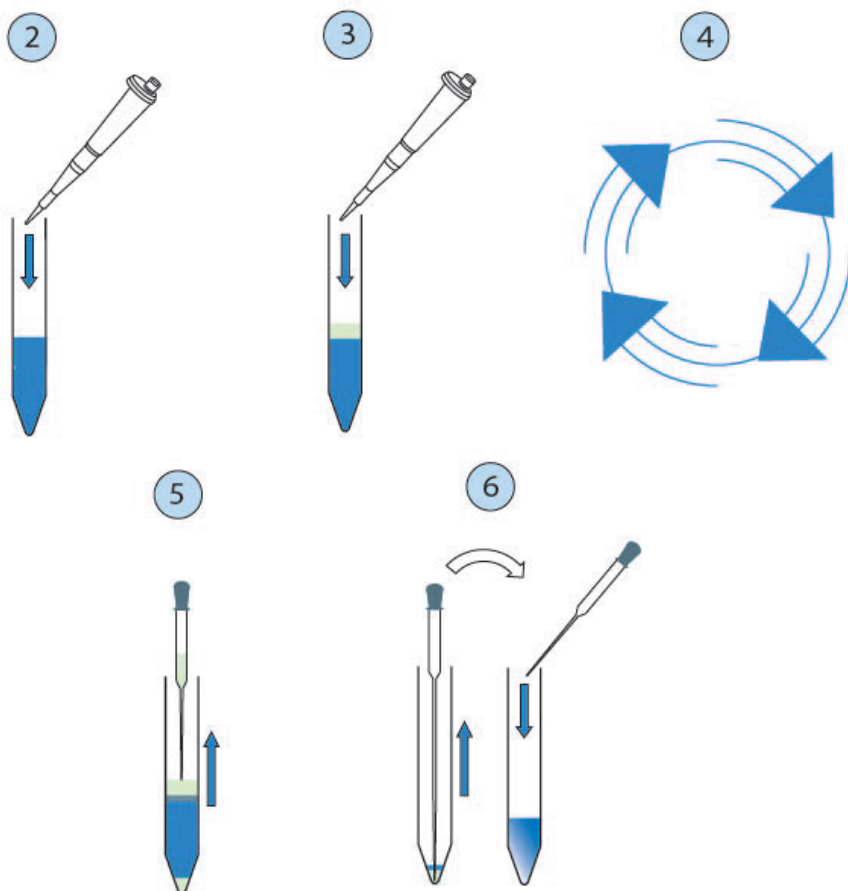
Reagents and equipment

- Semen extender - we recommend BotuSemen
- 50 or 15 mL conical centrifuge tubes
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Centrifuge with swing-out rotor.

Procedure

1. Dilute the ejaculate 1:1 with BotuSemen, BotuSemen Special or Botu Semen Gold.
2. Add 10-20 mL EquiPure at ambient temperature to a 50 mL centrifuge tube, or if smaller volumes, use a 15 mL tube and add 2 mL EquiPure.
3. Add an equal volume of extended semen to the tube using a Pasteur pipette or disposable syringe. Add the diluted semen slowly, making sure not to mix EquiPure and the semen. Important: The maximum total number of sperm must not exceed 3×10^9 .
4. Centrifuge at $400 \times g$ for 20 minutes (1600 rpm in a centrifuge with a radius of 140 mm), do not use the brake. If only one tube is to be centrifuged, balance the rotor with another 50 mL or 15 mL tube containing an equal volume of water.
5. After centrifugation, remove the ejaculate and the most part of the EquiPure using a pasteur pipette.
6. Using a new pasteur pipette, retrieve the pellet and resuspend in BotuSemen to a concentration of 50×10^6 sperm/mL.

Sperm recovery after selection with EquiPure largely depends on the initial sperm quality, but on average ranges around 40%.



Concentrating sperm prior to EquiPure treatment

Spermfilter

Semen can be concentrated using SpermFilter before centrifugation with EquiPure. In this case dilute the ejaculate 1:1 with BotuSemen and filter in SpermFilter (video demo www.botupharma.com.br). Then resuspend in BotuSemen (10 mL). Use EquiPure as described.

Centrifugation

Dilute the ejaculate 1:1 with BotuSemen and centrifuge at 600 x g for 10 minutes (1950 rpm in a centrifuge with a radius of 140 mm), do not use the brake.

Resuspend the pellet in BotuSemen (5-10 mL) and proceed using EquiPure as described above.

EquiPure prior to freezing sperm

Sperm selection by EquiPure is recommended for stallions with poor freezing tolerance.

After centrifugation with EquiPure as described above, resuspend the pellet in BotuCrio to a concentration of 200×10^6 motile sperm/mL.

EquiPure post thaw

EquiPure can also be used with frozen/thawed samples with low viability, motility or membrane integrity. In these cases the procedure is based on selecting a sperm population with the highest viability.

After thawing of the 500 µl-straws, add the same volume EquiPure as the volume of the thawed straws to a 15 mL centrifuge tube

Slowly add the thawed semen sample on top of the EquiPure, ensuring there is no mixing between the EquiPure and thawed semen.

Centrifuge at 400 x g for 20 minutes (1600 rpm in a centrifuge with a radius of 140 mm), do not use the brake. After centrifugation, aspirate the pellet.

The aspirated material should be resuspended in 4 mL BotuCrio.

Insemination is preferably performed at the end of the uterine horn on the site of ovulation.



Extenders

Background

The semen extenders from BotuPharma come in powder form. The advantage is that the extenders stay stable for three years. As is well known, antibiotics in solution break down and lose their efficiency. Penicillin especially is not stable in solution for more than a week or two.

Also, the extenders can be stored in room temperature until constituted in water.

Nidacon provides water with the powdered extenders. It is vital not to use any kind of water. It needs to be sterile and completely de-ionized not to disturb the composition of the extender. All extenders contain Penicillin K 1 g/l and Gentamicin 1 g/l.

All extenders can be stored at 2-8°C for up to three days after reconstitution in water.

Already reconstituted extender can be frozen and stored frozen for up to one year.

Presentation:

5 packets (5 x 8.5 g) including funnel for easier reconstitution in water.

The extenders are provided with or without PureWater.

BotuSemen

BotuSemen was developed after years of research and its current formulation allows the safe transport of sperm until the time of insemination. It is also used to dilute the ejaculate during the freeze-spinning process.

It can also be used to extend semen prior to using EquiPure.

Composition:

Skim milk powder UHT sterilized, sugars, amino acids, antioxidants and excipients.

Stability:

2 years in dry form, 1 year reconstituted and frozen, three days in refrigerator after reconstitution in water.

Preparation:

Add all content of the packet in 100 mL PureWater and shake very well.

Usage:

For transport of cooled semen, dilute to concentration of 30-50 million sperm/mL.

Extend 1:1 (v/v) and wait 15 minutes for insemination with non cooled semen.

BotuSemen Special

BotuSemen Special is especially developed for stallions with semen sensitive to the cooling process. Its composition is based on addition of essential lipids to maintain the stability of the sperm plasma membrane.

It can also be used to extend semen prior to using EquiPure.

Composition:

Skim milk powder UHT sterilized, sugars, amino acids, antioxidants, cholesterol and excipients.

Stability:

2 years in dry form, 1 year reconstituted and frozen, three days in refrigerator after reconstitution in water.

Preparation:

Add all content of the packet in 100mL PureWater and shake very well.

Usage:

For transport of cooled semen, dilute to concentration of 30-50 million sperm/mL.

Extend 1:1 (v:v), wait 15 minutes for insemination with non cooled semen.

BotuTurbo

BotuTurbo is developed to improve the mobility of slow moving sperm. It is highly recommended for insemination and transport of fresh semen from stallions that need more movement.

Composition:

Skim milk powder UHT sterilized, sugars, amino acids, antioxidants, pentoxifylline and excipients.

Stability:

2 years at room temperature in dry form, 1 year reconstituted and frozen, three days in refrigerator after reconstitution in water.

Preparation:

Add all content of the packet in 100 mL PureWater and shake very well.

Usage:

For transport of cooled semen dilute to concentration of 30-50 million sperm/mL.

Extend 1:1 (v:v), wait 15 minutes for insemination with non cooled spemen.

Highly recommended for reinforcement breeding (thoroughbred).

BotuSemen GOLD

BotuSemen Gold is based on casein which in high concentrations contributes to a delay in the sperm capacitation process, improving pregnancy rates.

It is can also be used to extend semen prior to using Equi-Pure.

Milk casein phosphoproteins protects sperm cells from seminal plasma proteins (HSP-1, 2) which occur in the seminal plasma.

Composition:

Casein, sugars, amino acids, antioxidants, cholesterol and excipients.

Stability:

2 years in refrigerator in dry form, 1 year reconstituted and frozen, three days in refrigerator after reconstitution in water.

Preparation:

Add all content of the packet in 100 mL PureWater and shake very well.

After dilution wait 30 minutes before use.

Usage:

In cases where centrifugation before cooling is necessary, resuspend to at least 100×10^6 spetz/mL. Use dilution table below:

Spermatic concentration	Dilution (v:v)
$< 90 \times 10^6/\text{mL}$	1:1 Centrifuge and resuspend to 100×10^6 spetz/mL
$90 - 120 \times 10^6/\text{mL}$	2:1
$130 - 160 \times 10^6/\text{mL}$	3:1
$170 - 210 \times 10^6/\text{mL}$	4:1
$220 - 250 \times 10^6/\text{mL}$	5:1
$260 - 300 \times 10^6/\text{mL}$	6:1

Freezing extender

BotuCrio

BotiCrio is the result of years of research and fertility trials at the state university of Sao Paolo, Brazil and is extensively tested in the field.

The extender contains the optimal components to protect cells from the damage caused by freezing and to protect their fertility after thawing.

BotiCrio contains a low concentration of glycerol, which in combination with one of several amides, results in better sperm integrity, sperm viability, better post-thaw total and progressive motility, as well as sperm velocity, compared to other commercially available extenders.

Studies have indicated that stallion sperm frozen in BotuCrio are more fertile compared with other extenders.

These in vitro results have been confirmed in field experiments which show higher pregnancy rates after insemination.

The higher pregnancy rates achieved with semen frozen in BotuCrio results in a higher pregnancy rate per cycle and less inseminations per pregnancy. This reduces the cost to breed a mare.

BotuCrio is today used for both good and poor freezing stallions in freezing centers in Australia, The Middle East, North and South America as well as in Europe.

Composition:

Sugars, antioxidants, amino acids, egg yolk, cryoprotectants and excipients and Gentamicine 0.5 g/l.

Stability:

1 year frozen. Three days in refrigerator after thaw.

Use:

BotuCrio is shipped and stored frozen. Thaw BotuCrio in 37°C water until completely thawed. Let rest on bench 5 minutes. Ensure that it is well in room temperature before use. The fat from the egg yolk must have time to completely dissolve, it can otherwise be seen as black debris. This however is completely harmless to the spermatozoa.

After semen collection:

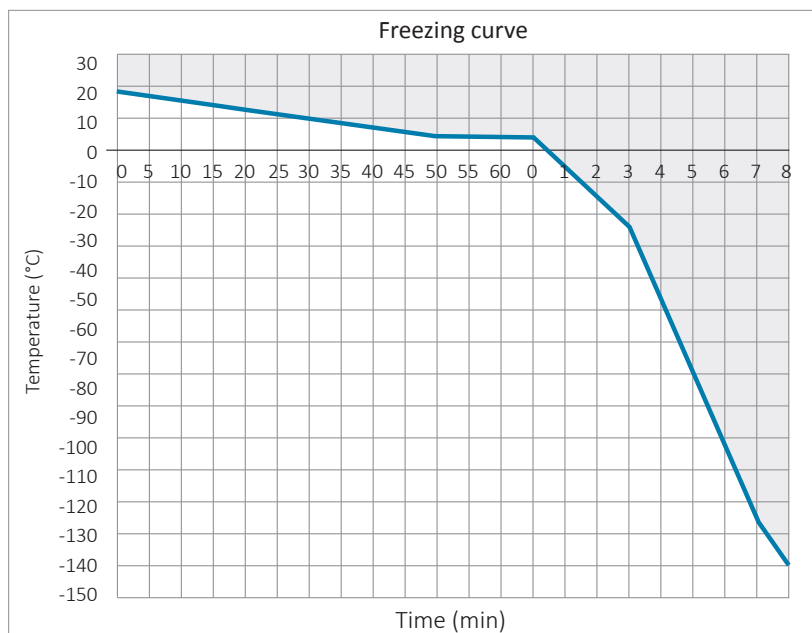
1. Determine sperm parameters: volume, motility and concentration.
2. Dilute the ejaculate with extender 1:1 (v:v). Use BotuSemen Gold for optimal results
3. Centrifuge at 600 x g for 10 minutes or with Red-Cushion at 1000 x g for 20 minutes.
4. Remove supernatant.
5. Resuspend pellet with BotuCrio to 200x10⁶ or 100x10⁶ sperm/straw.
6. Pack and seal straws.
7. Leave straws in refrigerator at 5°C for 20-30 minutes.
8. Add 6 cm (2.5 inches) of nitrogen into styrofoam box.
9. Put straws 3-6 cm (1.2-2.4 inches) above the liquid nitrogen surface for 20 minutes.
10. Plunge the straws directly into the liquid nitrogen.

Freezing machine:

1. Set machine to 5°C.
2. Add straws to machine and cool at 5°C for 20-30 minutes.
3. Start the cooling rate to -30-60°C/minute until at least -120°C.
- 4 Plunge straws directly into the liquid nitrogen.

Thaw straws in water batch at 37°C for 30 sec or at 46°C for 20 sec.

Using EquiPure pre or post thaw will increase survival and fertilization rates.



Red Cushion

A cushion fluid of high density used for semen centrifugation. It reduces damage from centrifugation through sperm damping over the RedCushion on the bottom of the tube. It produces less compacted pellets with high sperm recovery rate due to the possibility of working with intensity and time of centrifugation above those used in conventional protocols. Because it has a reddish coloration, it allows easy visualization of the spermatozoa layer and its removal.

Two different method can be used:

Usage (1):

1. Aspirate 3 mL of RedCushion with syringe and needle, and deposit at the bottom of the centrifugation tube.
2. Dilute the ejaculate with BotuSemen in the proportion of 1:1 v/v.
3. Incline the tube to 45° and slowly deposit the diluted semen on the wall with plastic Pasteur pipettes so that no mixing occurs between the semen and the RedCushion.
4. Centrifuge at 1000 x g for 20 minutes.
5. Using a pipette remove the sperm layer deposited on top of RedCushion.
6. Resuspend the sperm with BotuSemen or BotuCrio, according to the procedure to be held.

Usage (2):

1. Dilute the ejaculate with BotuSemen in the proportion of 1:1 v/v.
2. Insert the diluted semen into a centrifuge tube.
3. Using a new syringe and needles deposit 3 mL of RedCushion under the diluted semen.
4. Centrifuge at 1000 x g for 20 minutes.
5. Remove the supernatant.
6. Aspirate the formed layer over the RedCushion.
7. Resuspend the sperm with BotuSemen or BotuCrio, according to the following procedure.

Storage:

Store at 2-8°C. Shipped at room temperature.



SpermFilter

A more sensitive way of concentrating semen is Sperm Filter which is a synthetic membrane with a porosity that allows the passage of seminal plasma and bacteria, retaining only sperms. The filter eliminates the need of a centrifuge, being less harmful to sperm when compared to the centrifugation usually used to concentrate sperm.

Usage:

1. Carefully remove the filter from the package. Place the Petri dish on a surface heated to a temperature of 30-35°C.
2. Dilute the semen with BotuSemen and pour it into the SpermFilter.
3. To concentrate the sperm, gently swirl the diluted semen around the filter and repeatedly touch the bottom of filter on the bottom of Petri dish until only a slight amount of liquid is evident on the filter membrane.

4. Wash the filter with appropriate volume of either BotuSemen or BotuCrio extender. Repeat swirling motions to resuspend the sperm and pour it into a beaker. If sperm recovery is low due to smaller size of the sperm, it may be necessary to refilter the ejaculate.

The SpermFilter can be reused for the same stallion at least 10 times. Wash it with sterile distilled water and dry at 37°C.



SpermCatch™

SpermCatch is a viscous solution designed to modulate sperm motility in a physiological manner. The preparation is supplied ready for use in a sterile isotonic salt solution.

SpermCatch is an alternative to PVP (polyvinylpyrrolidone) which today is the most common substance used for slowing down sperm prior to ICSI. However, PVP has been reported to cause problems, such as damaging the sperm plasma membrane. It may also interfere with sperm nucleus condensation.

SpermCatch is a solution without PVP, instead it contains

hyaluronic acid which is a natural component.

The sperm is injected into the oocyte, hyaluronic acid is preferable to plastic (PVP).

Components:

Salts, Bicarbonate, Sugars, EDTA, HEPES, Human Serum Albumin, Hyaluronic Acid.

Stability:

1 year at room temperature.

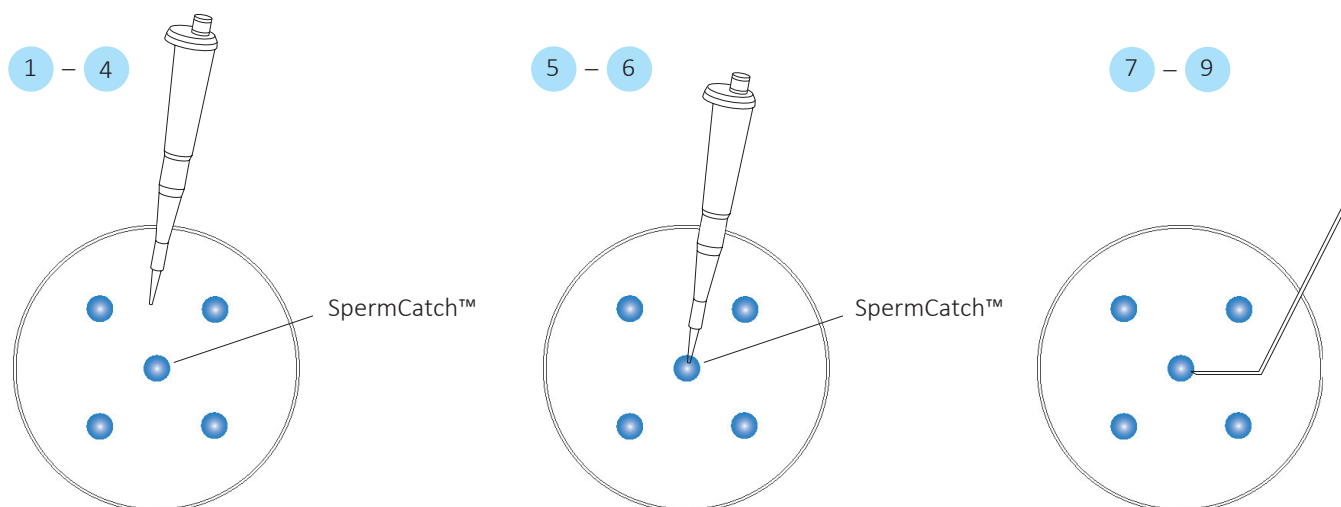
Reagents and equipment

SpermCatch
NidOil
Injection media

Sterile pipettes
ICSI equipment
Petri dish

Procedure

- Place a 10 μ L drop of SpermCatch in the middle of a petri dish.
- Place 4 drops of 10 μ L injection media around the SpermCatch drop in the petri dish.
- Immediately cover the drops with NidOil.
- Incubate for 30 minutes in CO₂ environment at 37°C.
- Add 1 μ L of prepared sperm suspension to the middle of the SpermCatch drop.
- Incubate for 10 minutes in CO₂ environment at 37°C.
- Fill your injection pipette with SpermCatch to avoid the sperm sticking to the inside of your pipette. It will also help you to make a controlled injection.
- Immobilise the individual sperm by using the injection pipette to "nick" the sperm tail.
- Aspirate the immobilised sperm.
- Move to one of the oocyte droplets. Focus on the oocyte and position the oocyte with the holding pipette. Bring down your injection pipette and inject the sperm. Make sure that the oolemma is broken before you expel the sperm.



NidOil™

NidOil is a paraffin oil which has been specifically chosen and then treated in our production facilities to ensure that its purity and handling characteristics are suitable for using as an overlay when culturing gametes and embryos.

NidOil does not require washing before use, and it is neither too sticky nor too viscous, to facilitate pipetting.

Many questions have been raised lately to whether the oil that is used for covering an embryo culture can actually damage the embryo. All oil batches today from different manufactures are tested for sterility, endotoxins and a mouse embryo assay showing blastocyst development. This is apparently not enough, since damage to cultures has been observed with an appro-

ved batch of oil. One answer could be peroxidation of the oil which has been investigated in several publications and found to be harmful to fertilisation and embryo development when over a certain level.

Our stringent quality assurance control helps maintain our standard for low endotoxin and peroxide levels and also ensures our products are free from microbiological contamination. There have been several reports of paraffin oils becoming embryo-toxic after exposure to light on the laboratory bench. As a precaution against any possible light-induced changes, NidOil is packaged in amber, screw-top bottles.

Recommendations before use

NidOil should be equilibrated in the same way as the culture medium before use to avoid differences in

temperature and gaseous content between the components of the culture system.



Embryo transport media

BotuEmbryo

BotuEmbryo is a media for maintenance and transport of embryos. Its packaging does not require the use of syringes and needles. It has an innovative formulation based on scientific studies carried out by national and international researchers.

Usage:

Transfer embryo to a Petri dish containing BotuEmbryo.

Storage and stability:

Transport temperature: Ambient for up to 3 days.

Storage temperature: 1 year at 2-8°C

BotuEmbryo is highly stable, allowing the embryo transport at ambient or refrigerated temperature in appropriate containers for up to 24 hours.

Performance pH: 7.0-7.4

Osmolality: 290-310

Components

Amino acids, vitamins, sugar, phenol red, hepes, streptomycin amphotericin, bovine serum albumin and preservatives.



Sperm VitalStain™

Sperm VitalStain is an optimised staining technique for assessment of Equine sperm vitality and morphology, which are two of the basic tools used in semen analysis.

The Sperm VitalStain technique for vitality assessment is based on the principle that dead cells (i.e. those with damaged plasma membrane) will take up the eosin and stain red. Nigrosine is used as a counterstain to facilitate visualization of the unstained (white) live cells.

Sperm VitalStain can also be used to highlight details when assessing normal and abnormal sperm (morphological examination).

Sperm VitalStain can be stored at room temperature for up to 2 years from production date, ensuring minimal wastage. It should not be stored in refrigerator.

Reagents and equipment

Light microscope (40 – 100 x magnification)
Pipette

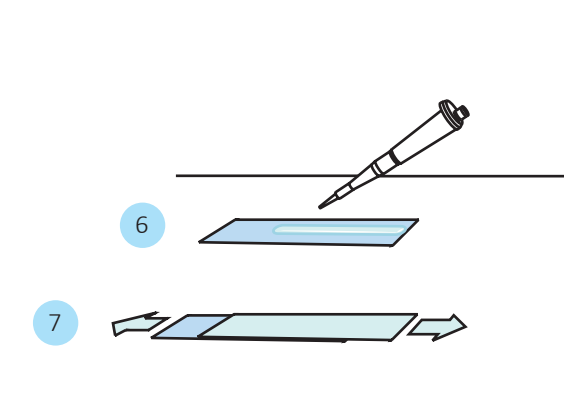
Microscope slides
Test tube

Procedure

1. Shake the bottle of Sperm VitalStain before use.
2. Take an equal amount of Sperm VitalStain and the sperm sample (eg. 50 μ L SVS + 50 μ L sample). Use for example an eppendorf tube.
3. Mix well.
4. Leave for 30 seconds at room temperature.
5. Prepare a slide using your conventional method or use the method recommended by Nidacon.
6. Transfer a 20 μ L drop onto a labelled microscope slide with a pipette, making a string/line of fluid in the middle of the slide.
7. Cover this slide with a second microscope slide and, when the drop is evenly spread between the two slides, pull them apart from each other horizontally. You then have two good slides.
8. Air dry the two slides and examine. If you want to store for later use, mount the slides with DPX or equivalent mountant, and a cover slide.
9. Examine using a bright field 40 x objective or a 100 x objective under oil immersion.
10. Count 200 sperm, the white (unstained) are classified as alive and the red or pink are classified as dead. Sperm coloured only at the neck region are classified as alive.

Tips

- The 100x objective with immersion oil will give you a very clear picture of stained versus unstained sperm.



References

- Equine Semen Cryopreservation, Sanches R et al, Equine Assisted Reproduction, ISBN 978-958-59883-1-6, Section 3, 2019
- Semen Processing for the Subfertile Stallion, Varner D.D et al, Journal of Veterinary Science, Vol 28, no 11 (2008)
- Increasing Stallion Fertility, Quarterback news, December 15, 2013. pp. 78-80
- Use of a Single-layer Density Centrifugation Method Enhances Sperm Quality in Cryopreserved-Thawed Equine Spermatozoa, Stoll, A et al. Journal of Equine Vet Science, 2012
- Insemination Doses: How Low Can We Go? Brinsko S P et al, Theriogenology 66 (2006) 543-550
- Total RNA isolation from stallion sperm and testis biopsies, Pranab j Das et al, Texas A&M, Theriogenology 74 (2010) 1099-1106
- Does the Microbial Flora in the Ejaculate Affect the Freezeability of Stallion Sperm?, C Ortega-Ferrusola et al Reprod. Dom Anim 44, (2009) pp 518-522
- Use of a Silane-Coated Particle Solution to Enhance the Quality of Ejaculated Semen in Stallions, Varner D.D et al, Theriogenology, Volume 58, Issue 2, Pages 317-320
- Pregnancy rates in mares inseminated with 0.5 or 1 million sperm using hysteroscopic or transrectally guided deep-horn insemination techniques, S.S Hayden, DD Varner et al., Theriogenology 2012.
- How to improve the freezeability and fertility of stallion semen using a silanated silica-particle solution (EquiPure™), Samper, JC 2010
- Evaluation of Cryopreserved-thawed Stallion Sperm Before and after Density Gradient Centrifugation with Silane-coated Silica Particles (EquiPure®), Stoll A, et al. Theriogenology 70 (2008) pp. 576-591.
- Advanced Methods for Handling and Preparation of Stallion Semen, Loomis et al, Anim Reprod Sci. 2006 Feb;91(3-4):237-47
- Effective Removal of Equine Arteritis Virus From Stallion Semen, Geraghty, R.M. Equine Veterinary Journal, Volume 38, Number 3, May 2006, pp. 224-229(6)
- Effect of Centrifugal Fractionation Protocols on Quality and Recovery Rate of Equine Sperm, A.J Edmont, S.P Brinsko, DD Varner et al. Theriogenology [0093-691X] Edmond year:2012 vol.:77 no:5 p:959
- Effect of density-gradient centrifugation on quality and recovery rate of equine spermatozoa, Edmond A.J, Varner D.D et al. Abstracts, Animal rep Science 2008
- Simple and economic colloidal centrifugation protocols may be incorporated to the clinical equine sperm processing procedure. Gutierrez-Cepeda et al. Animal Medicine and Surgery Dept, Vet Faculty, Madrid, Spain.
- Effect of single layer colloidal centrifugation prior to equine semen cryopreservation on sperm
- chromatin integrity. Gutiérrez-Cepeda, L.*a; Fernández, A.a; et al, Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria
- The effect of two pre-cryopreservation single layer colloidal centrifugation protocols in combination with different freezing extenders of the fragmentation dynamics of thawed equine sperm DNA, Gutierrez-Cepeda et al. Acta Veterinaria Scandinavica 2012, 54:72
- Effect of non-sperm cells removal with single-layer colloidal centrifugation on myeloperoxidase concentration in post-thaw equine semen, Ponthier, J, et al. Theriogenology 80 (2013) 1082-1087
- Effect of density gradient centrifugation with three different media on stallion semen quality, Sabatini, C et al. Journal of Equine Vet Science 34 (2014) 77
- Recovery rate and sperm quality after centrifugation of stallion sperm in different gradient concentrations Love, CC et al, Journal of Equine Vet Science 34 (2014) 63
- Total RNA isolation from stallion semen sperm and testis biopsies, Das J Pranab, Varner DD et al. Theriogenology 74 (2010) 1099-1106
- Freezing of stallion epididymal sperm, Papa et al, Animal Reproduction Science 107 (2008) 293–301
- Replacing egg yolk with soybean lecithin in the cryopreservation of stallion semen, Papa et al, Animal Reproduction Science 129 (2011) 73– 77
- Influence of Semen Storage and Cryoprotectant on Post-thaw Viability and Fertility of Stallion Spermatozoa, Melo et al, 2007 Elsevier Inc.
- Comparison between three extenders for cooling of stallion semen before freezing, Alvarenga et al, (Abstract) doi:10.1016/j.anireprosci.2008.05.117
- Post-thaw characteristics and fertility of stallion semen frozen in extenders with different Cryoprotectants, J.C. Samper, (Abstract) Animal Reproduction Science (2008)
- Comparison between two extenders to cool equine Semen 24 hours before Freezing, (Abstract) Vetrenaria e Zootecnia.
- Effects of three cryopreservation systems on longevity of stallion sperm after thawing, D. F. Pasquini et al, Reproduction, Fertility and Development 20(1) 123 – 124
- Control Methods and Evaluation of Bacterial Growth on Fresh and Cooled Stallion Semen Neto et al, JEVS, v 35, p 277-282, 2015
- Advances in Stallion Semen Cryo Preservation, Alvarenga et al, America-Equine Practice, v. 32, p. 521-530, 2016.
- Effect of Removing Seminal Plasma Using a Sperm Filter on the Viability of Refrigerated Stallion Semen, Alvarenga et al. Journal of Equine Veterinary Science (2012) 1-4
- A new method to concentrate equine sperm, Alvarenga et al Animal Reproduction Science 121S (2010) S186–S187
- Comparison of Cushioned Centrifugation and SpermFilter Filtration on Longevity and Morphology of Cooled-stored Equine Semen, Roach et al, Veterinary record, v. 178, p 241-241, 2016



Product Manager
Anna Niläng
anna@nidacon.com
Phone +46-31-703 06 38



Marketing KAM
Mauricio Lucena
mauricio@nidacon.com
Phone +46-31-703 06 39



Logistics
Dennis Johansson
dennis@nidacon.com
Phone +46-31-703 06 37



Flöjelbergsgatan 16 B • S-431 37 Mölndal • Sweden
Phone +46-31-703 06 30 • Fax +46-31-40 54 15
contact@nidacon.com • www.nidacon.com