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SpermNotes®

High Efficient Boar Semen Production

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Stud and laboratory design

Stud design

A boar stud is the central point of a pig breeding organization. The main aim of a boar stud is to produce liquid genetics (=boar semen) with highest quality and efficiency.

To fulfill these requirements the boar stables and the semen production lab have to be well designed. Figure 1 shows a boar station for 100 to 200 boars with all necessary buildings. Each of the stables (No. 1) can host up to 100 boars.

Biosecurity is an important issue and therefore, any possible direct or indirect fomites should be kept away from the valuable animals. The picture shows a double fence as a barrier for wild animals, people and vehicles that should not enter the facility. Only clean and disinfected vehicles should pass the outer barrier. Only people working in the station and occasional visitors should enter the inner barrier which allows access to the working areas.

Any other traffic of people and vehicles should be prevented as far as possible.

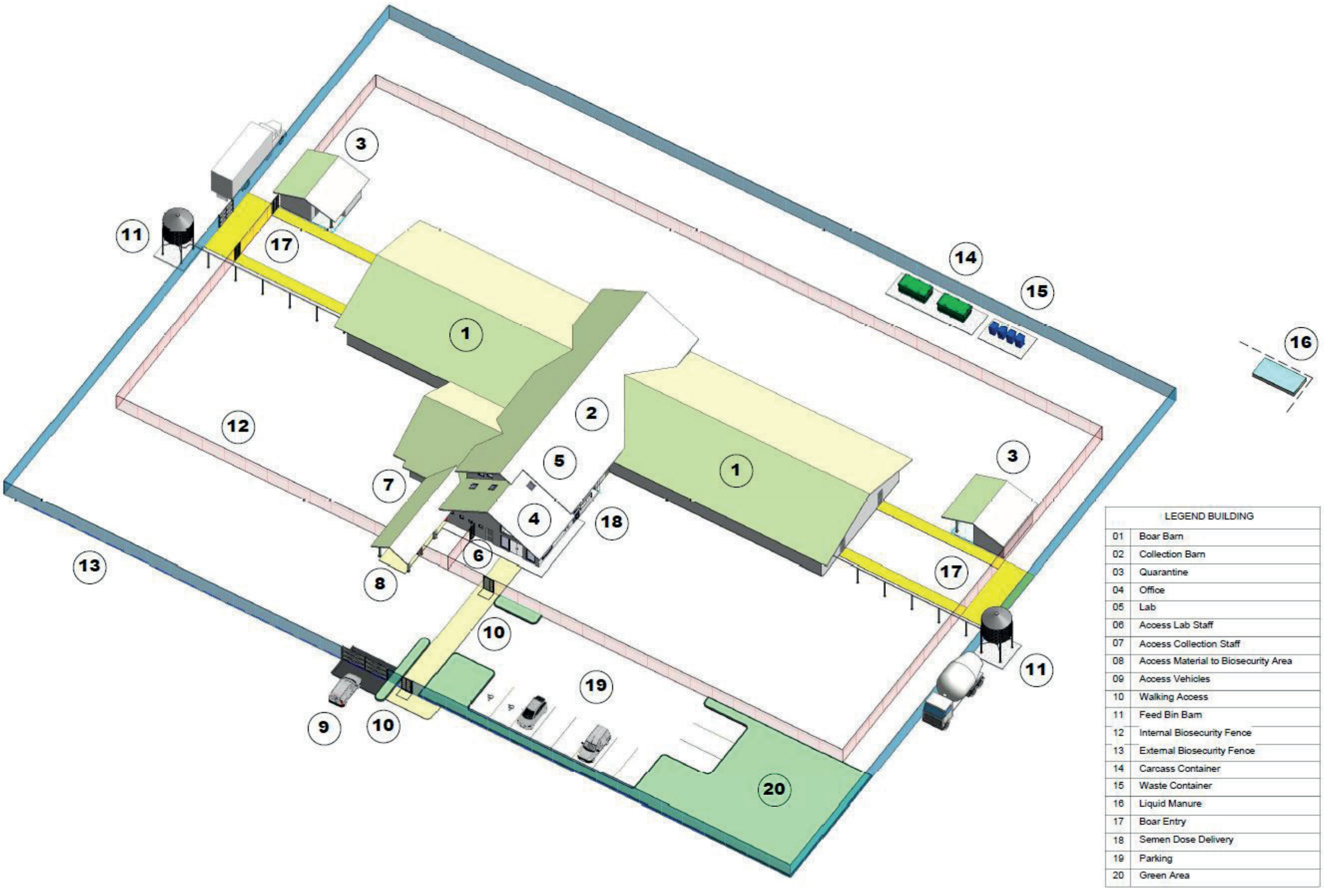


Figure 1: General overview boar stud with 100-200 boars

Working areas in a boar stud

Figure 2 shows more details of a boar stud, especially the working areas.

The yellow section is the area for semen collection (partially visible) and the working and preparation area for the staff working in the stable.

The blue area is the production laboratory, including the rooms for preparation and storage of material. It is very important, that both areas are separated from each other. People working in the stable should not enter the laboratory and vice versa. There should also be no crossings of ways during work. So both areas must have a separated entrance, including showers. This is important to assure a high level of hygiene especially in the laboratory. The only direct connection between laboratory and stable is the sluice where the collected boar semen is brought into the laboratory.

The orange area is dedicated for office use. People working here should neither have direct contact to the stable nor to the laboratory. The cooled semen doses leave the boar stud production facilities through a sluice from the cooling room to the office.

With the installment of these barriers, the animals and the laboratory are protected in a secure way against fomites.

Lab Area

No.	Room Name	Area
4	Access Lab	2.4 m^2
5	Women Changing Room + Shower	5.4 m^2
6	Men Changing Room + Shower	5.5 m^2
7	Storage of Lab Deliveries	9.5 m^2
8	Cleaning + Sterilization	4.8 m^2
9	Cool Room 17°, Packing	6.5 m^2
17	Machine Room	11.9 m^2
25	Hallway Lab	16.4 m^2
26	Toilet	2.1 m^2
27	Break Room Lab	7.8 m^2
28	Changing Lab Shoes + Coat	1.6 m^2
29	Lab : Evaluation, Dilution, Procesing	25.4 m^2
Total general		99.3 m^2

Collection Area

No.	Room Name	Area
10	Women Changing + Shower Room	5.2 m^2
11	Men Changing + Shower Room	5.3 m^2
12	Hallway Collection Room	17.7 m^2
13	Toilet	2.2 m^2
14	Wardrobe	0.8 m^2
15	Break Room Collection	7.3 m^2
16	Storage Collection Material	8.2 m^2
18	Changing Collection Shoes + Coat	3.6 m^2
30	Collection Barn	98.4 m^2
Total general		148.8 m^2

Office Area

No.	Room Name	Area
1	Reception	21.6 m^2
2	Access to Obs.	4.8 m^2
3	Public Toilets	2.3 m^2
24	Observation Room (2nd Floor)	43.0 m^2
Total general		71.7 m^2

Figure 2: Building plan of laboratory, collection area and office area of a boar stud

Laboratory design

The boar semen laboratory in figure 3 is organized according to the optimal workflow of semen production. The ejaculates, that were collected from the boars in the collection area enter the laboratory through the sluice and are first weighed (3) to determine the ejaculate volume. The ejaculates are kept warm during the analysis (4). The analysis of the semen proceeds with assessment of motility and concentration with a microscope and a CASA system like AndroVision® (5). After entering the data in a laboratory management software like IDEE, the boar semen is diluted (6,7). In the next step, the ejaculates are filled into tubes by a boar semen filling machine like the MiniBSP (8,9). A trolley (10) helps to collect and organize the filled tubes. After filling, the tubes are either stored at room temperature or brought into the cooling/packing area at 16°C (11), depending on the laboratory working protocol. In the cooling room, the semen tubes are stored and packed for delivery to the customer.

	DESCRIPTION
01	Bench Top Incubator
02	Semen Storage Unit
03	Scale for Weighing the Ejaculate
04	Warming Plate
05	Microscope + 2 Screens for Semen Analysis + Lab Data Management
06	Extender Tank
07	Smart Dispenser for Semen Extension
08	Waiting Area for Extended Ejaculates
09	MiniBSP
10	Trolley
11	17°C Storage
12	Semen Dose Delivery
13	Table
14	Withdrawal of Packaged Semen from Office
15	Withdrawal of Packaged Semen from Outside
16	Pass through Window for Ejaculates
17	Incubator
18	Stainless Steel Bench
19	Sink
20	Cupboard

Figure 3: Design of a boar semen production laboratory

Modern boar semen production laboratories

High quality semen doses provide fertile sperm cells and avoid the transmittance of pathogens into the female genital tract. Strict hygienic measures at all stages of semen collection and processing are therefore fundamental in order to minimize the bio burden of extended semen. In addition, a high level of automation minimizes the risk of contamination during semen production.

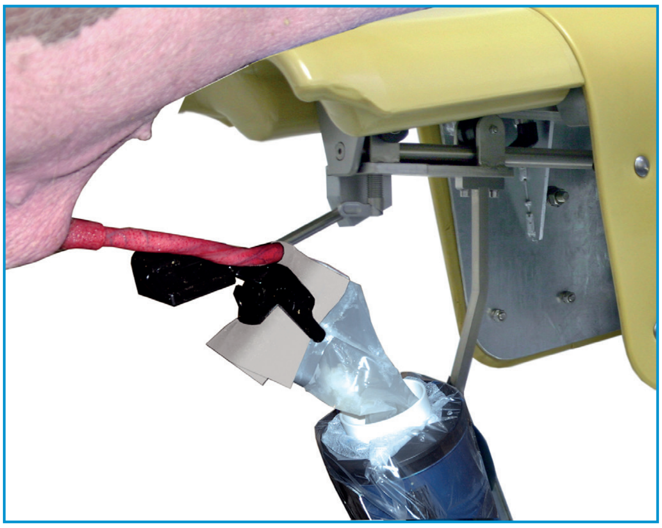


Figure 4: BoarMatic - boar semen collection system

Figure 4 shows the BoarMatic semi-automatic semen collection system. The boar dummy with an integrated mechanism for automatic semen collection includes a slide underneath the dummy with a holder for the semen collection cup and a clamp for the artificial cervix (AC). The single use AC is a true copy of the porcine cervix in texture and surface. A tear away inner pouch to collect the first sperm free jets of the ejaculate and a sheath leading the ejaculate into the semen collection cup are integrated in the AC. As shown on the picture the boar's penis is hold in a closed system. The semen is guided through the AC into the BlueBag. The closed system minimizes the possibility of fomites getting into the ejaculate during semen collection.

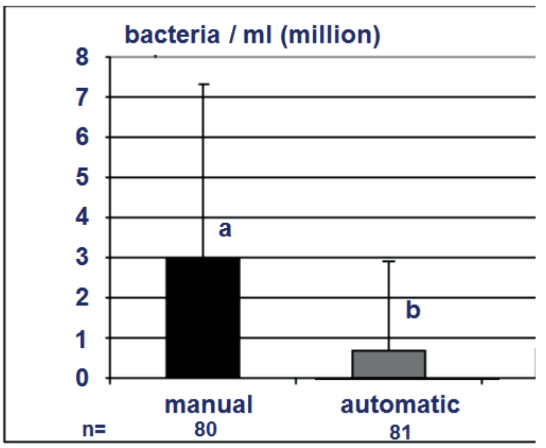


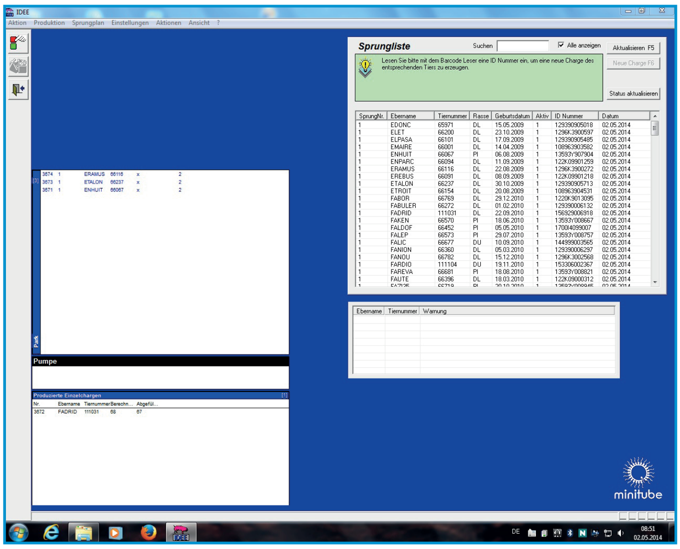
Figure 5: Bacterial content in native boar semen after manual or automatic semen collection

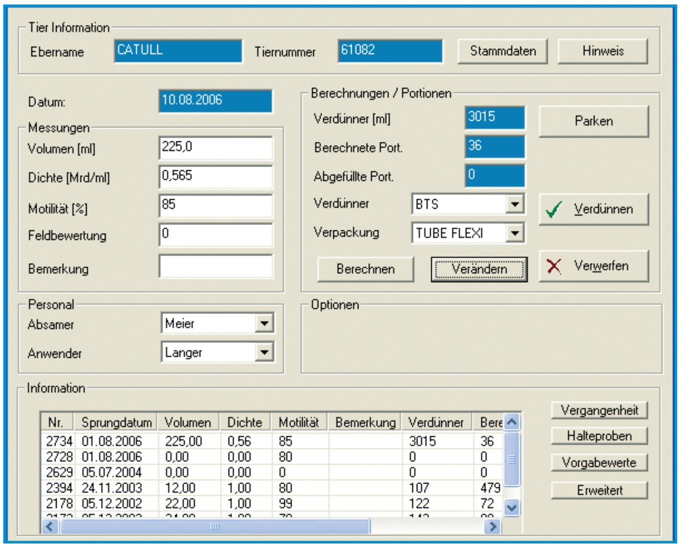
Figure 5 shows the bacterial content in boar ejaculates comparing manual and automatic semen collection.

Minitube' BlueBag is the complete one-way solution from semen collection to packaging and fits perfectly with BoarMatic. It has an integrated filter that is removed after collection together with the gel phase of the boar ejaculate. The collection cup can be prepared very quickly and the filter and inner surface of the BlueBag are kept contamination free. The BlueBag can be used for manual collection and semen collection with BoarMatic.

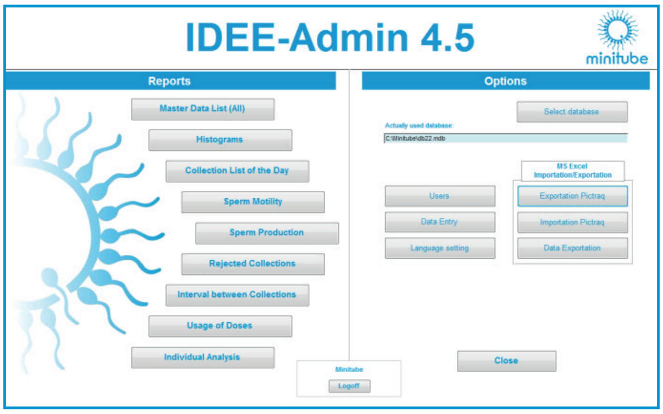
Figure 6: Universal semen bag (BlueBag) for boar semen collection

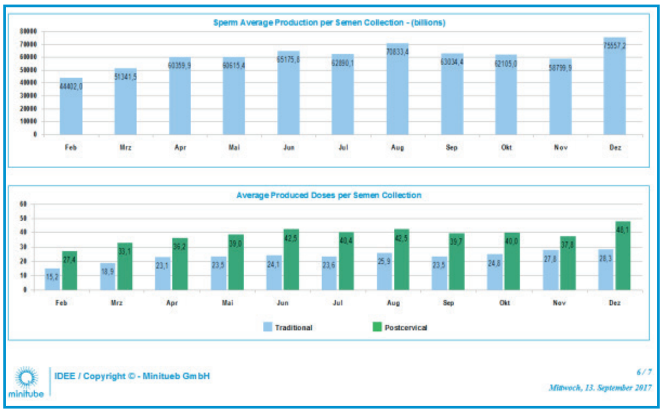
The first step after semen collection is the assessment of semen quality. Management software solutions for semen handling and objective assessment prevent mistakes due to human error.
The IDENT software contains collection planning, the identification of boars via electronic ear tags, the electronic identification of semen collection staff and the print-out of labels with boar identity and bar code. The software makes ejaculate identification very easy and prevents the risk of confounding ejaculates. IDENT can seamlessly be integrated in the laboratory management software IDEE-Production. IDEE-Production automates and links ejaculate analysis including record keeping of holding samples as well as extender dispensing and semen packaging in tubes. IDEE guarantees quick and precise ejaculate data management.

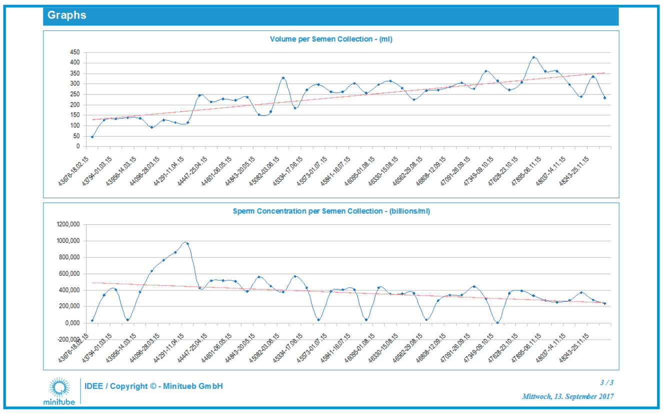




IDEE-Admin serves as a reporting tool for IDEE-Production and is an integral part of the IDEE software suite. Figures (9-11) show IDEE-Admin example reports.







Figures 7-11: IDENT and IDEE boar semen production software

Other equipment of the semen production line can also be integrated into the laboratory management software. AndroVision® is a CASA system for highly efficient automated and computerized semen analysis. AndroVision® performs the classic CASA analysis with more advanced assays for sperm functionality. The results of all measurements obtained with AndroVision® can be included in the IDEE ejaculate file.
AndroVision® can simultaneously measure semen concentration, motility, percentages of proximal and distal plasma droplets and bent tails. All these parameters can be included in the calculation of semen doses in order to optimize the quality of the produced semen. CASA systems are developing to a standard in semen analysis, as these measurement systems minimize the human influence (=error) on the semen analysis.

Figure 12: AndroVision® – for integrated sperm functionality analysis

Also the further processing of boar semen can be controlled with IDEE. The Smart Dispenser consists of an electronic control unit and a balance and is interfaced with a pump. It allows the highest level of security and precision for the process of semen dilution.

Figure 13: Smart Dispenser - high precision boar semen dilution

MiniBSP - an automatic filling machine for boar semen - is a space saving table model and is suitable for the processing of up to 950 semen tubes per hour. It features an integrated tube magazine and a control of the filling level by electronic weighing cells, with a filling accuracy of +/- 1ml per tube.
Of course the MiniBSP can also be included and controlled in the IDEE management software.

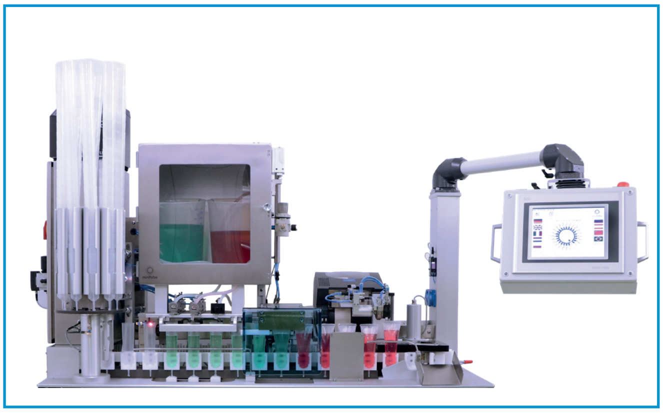


Figure 14: MiniBSP - automatic boar semen filling and sealing machine

High quality sperm production in Germany

Germany is one of the biggest pig producers worldwide. In 2017 Germany had a stock of 27.1 million pigs altogether (1.9 million of them were sows). The average number of weaned piglets per sow and year is 29.0. The top farms in Germany reach about 35 weaned piglets per sow and year.

To make this excellent numbers possible, a high level of semen production for artificial insemination is a prerequisite. In average nearly 1900 semen doses are produced per boar and year in Germany. In the USA this number only reaches 1259 produced doses per boar and year (Riesenbeck, 2011).

The main reason for the high efficient boar semen production in Germany is the very high level in automation and standardization in all semen production laboratories. German boar studs are organized in a national organisation (BRS e.V.) in Bonn. The member organizations follow a strict self-commitment of a minimum standard in boar semen quality.

For example, all boar ejaculates that are used for semen production must fulfill the following requirements:

In addition to these requirements for native ejaculates, semen doses produced by a BRS-Member must have a minimum of 1.8 billion sperm per tube, a 80ml dose volume and 65% sperm motility after 3 days of storage.

In order to keep this high standard, all BRS boar studs have an ongoing quality monitoring program. This includes audits by independent scientists, independent control of semen quality by national reference laboratories, regular staff training courses, research promotion and many other quality assuring measurements.

Attribute	Minimum requirement
Colour	Gray white, white, yellow white
Consistency	Milky or whey-like
Admixture (urine, blood, pus)	None
Pollution (excrement particle, hair)	None
Smell	Neutral
Volume without Bulbourethral gland secretion [ml]	100
Sperm concentration [10^6/ µl]	Boar age: ≤ 9 months: 0.150 > 9 months: 0.200
Total sperm cells [10^9/ejaculate]	Boar age: ≤ 9 months: 15.0 > 9 months: 20.0
Sperm motility [%]	70
Sperm motility [%] at 72 h conservation	65
Morphological abnormalities (including plasma droplets) [%]	≤ 25
Spem cells with changes of the head [%]	≤ 5
Sperm cells with changes of the head caps [%]	≤10
Sperm cells with plasma droplets [%]	≤15
Spem cells with bent tails [%]	≤15
Other morphological abnormalities [%]	≤15
Germ content	No germs that are pathogenic to humans and animals

Figure 15: Minimum requirements for boar ejaculates that are used for semen production

Water quality in the boar semen production laboratory

Purified water is needed in semen production for two purposes:

- Preparing extenders
- Cleaning and rinsing glassware, filling water bath and vapor sterilizer

Tap water doesn't contain only pure water (H2O), it contains also minerals, like calcium carbonate, magnesium carbonate, nitrates, phosphates and other ions. It may contain even variable levels of chemical waste, organic compounds, microorganisms and endotoxines. In many areas, low levels of chlorine and fluorine are added to tap water for human health prevention.

In order to obtain appropriate tap water for semen laboratory use, water must go through a purification process. Particularly semen doses require a very high grade of purified water.

Purification systems for water

1. Distillation

With the distiller, water is vaporized by heat, condensed and collected as distilled water. Distilled water is practically sterile and to a high degree deionised. However, volatile organic compounds will remain in the distillate. For water distillation, mono- or bi-distiller equipments are required and frequently a previous step of deionisation is used. "Hard" water, with a high content of calcium carbonate, has to be deionised before entering the distiller. Otherwise, the distillation process will be very time consuming and the tubing inside the distiller must be de-scaled frequently. The process of distillation requires an important additional amount of tap water for cooling. Distillation is an excellent option when a relative small water volume is needed for preparing extenders, i.e. up to 30 liters each day. The distiller has to be properly serviced and quite often de-scaled. A bad serviced distiller doesn't produce properly pure water. For medium or large boar semen production laboratories, the distiller is usually not rapid enough.

2. Demineralization

The demineralization, also called de-ionization, removes ionic compounds from the water, such as Ca2+ or Mg2+. It works with resin, a component of small particles electrically charged, contained in cartouches where water flows through. Ionic components are retained in the resin. After some months (1-3, depending on the system and the needs of purified water), resins have to be replaced or recycled. The process of purification is pretty rapid and requires little energy. It is important to have a conductivity measuring device connected to the system, indicating the moment the process begins to get ineffective. This conductivity measuring device has to be checked regularly and, when the measured value is exceeding, the cartouche has to be replaced immediately by a new one. Deionized or demineralized water is not sterile. Bacteria contained in the water will pass through the process. Resins are not sterile and bacteria grow rapidly inside the cartouches, which may deliver an even more contaminated product than tap water. For this reason, it is important to use the same cartouche for no more than 3 months, even when the measured conductivity remains below the limit. In order to control bacteria, an additional treatment of the demineralized water is necessary. It can be done by distillation or with a bacterial filter (pore size 0.2 µm) or by using ultra-violet light. The demineralization combined with a bacterial control method is a very convenient choice for laboratories having a very high quality of tap water, a good supply of cartouches or a recycling system and the capacity of sustaining continuous costs. This solution is especially recommended for boar semen production laboratories needing high volumes of purified water, i.e. more than 50 liters each day.

3. Reverse osmosis

In reverse osmosis purification systems, pressure is applied to a container with tap water pushing the tap water through a semi-permeable filter which retains the undesired components. After passing the filter, water comes out purified, being retained by the osmotic pressure up to 98 % of solved components. However, the contained gases are not retained. Reverse osmosis is quite efficiently removing bacteria. If tap water is "hard", having high conductivity, reverse osmosis is not efficient enough in demineralizing, making it necessary to install a second process. For this reason, reverse osmosis is frequently combined with demineralization and ultra-filtration. Reverse osmosis needs an important additional volume of water for flushing the filter-membrane. Only 20 to 30 % of the used water is purified. Reverse osmosis is frequently used in semen laboratories with high requirement of water volume (100 liters or more each day). Combination of demineralization and reverse osmosis is very efficient, frequently accompanied by UV light treatment. The latter is used to sterilize the product water.

The most utilized and efficient system is the combination of reverse osmosis, deionization, filtering with coal filter and UV light exposition.

The chosen method for water purification will depend on the tap water quality of the AI center and the volumes of purified water needed each day. In every case, quality parameters have to be fulfilled.

For classifying quality of purified water, the ASTM system has been adopted, which recognizes 4 different types of water (Figure 16).

Parameter	TYP I	TYP II	TYP III	TYP IV
Conductivity (micro siemens/cm)	0.056	1.0	2.5	5
Electric resistance (Mega Ohm/cm)	18	1.0	4.0	0.2
Bacterial growth (UFC/ml)	0 (A)	10 (B)	100 (C)	100 (C)
TOC (ppb)	10	50	200	-
Sodium (ppb)	1	5	10	50
Chloride (ppb)	1	5	10	50
Silicates total (ppb)	3	3	500	-
Heavy metals (mg/l)	0.01	-	-	
Endotoxins	0.03	0.25		

- ASTM (American Society for Testing and Material)

Figure 16: Classification of water types

For preparation of semen extenders, water quality following ASTM Typ II is recommended. It has to comply with the following minimum requirements:

- Conductivity: below 5 µS/cm. Conductivity is a value showing presence of ions or salts in the water. If the value is too high, water will damage the semen, because osmolarity of the medium is increased. Calcium has to be completely eliminated, since percentage of capacitated sperm will increase after time. Water with a higher conductivity (up to 20µS/cm) may be used, if compensated with higher concentration of sperm cells in the seminal doses.
- Bacterial content: ideally 0 and maximum 1 CFU (=1 colony forming unit) per 10 ml. Bacterial content is harmful and tends to proliferate in the semen extender, which is an excellent medium for bacterial growth. Bacteria often motivate the reduced survival of extended semen, the reason why only a very low bacterial content in the water is acceptable. However, specific pathogenic organisms (bacteria and virus causing specific diseases in animals) have to be excluded. Presence of endotoxins must be low, because they are highly toxic for sperm cells.
- TOC (total organic components): maximum 50 ppb. This is a parameter for the total content of organic carbon in water. TOC reflects the presence of organic compounds produced by industrial, household and agriculture chemical waste. This factor should not be exceeded, being a good indicator for the general quality of water.

The requirements for water used for rinsing glassware or other material getting in contact with semen are lower. For this purpose demineralized water will be sufficient. However, rinsing has always to be done very thoroughly, in order to eliminate minerals or detergents adhered to surfaces which could get in contact with semen or extenders.

It is important to remember, that quality of purified water deteriorates with the time, depending on the storing system. As a norm, storage for more than one week is not recommended.

Recommendations

1. Before deciding about the system for purification of water:

- Tap water analysis: calcium carbonate contents, bacteria, organic contamination, conductivity and osmotic pressure.
- Definition of requirements: How much purified water should be produced each day? In which time period will this volume of water be needed?

2. Care of purified water: purified water may easily deteriorate before it is used. Immediately after production it may flow through a non sterile tubing, containing even growing bacterial colonies as a biological film that constantly contaminates the water. This happens frequently and is particularly critical in relatively old systems. Containers and bottles for purified water storage are frequently not sterile, especially if they are not kept close. If exposed to light, bacteria and algae may proliferate. Some bacteria, for example, the pseudomonas group, can use the plastic compounds of tubing for their metabolism. Purified water should not be stored, but used immediately for best. Tubing, faucets and containers should be sterilized frequently.

3. Alternative: buying water. If there is no water purifying system available, the best choice is purchasing purified water. However, deionised water for domestic use is not sufficiently pure for semen extender preparation. Purchased water should be sterile, deionised (max. conductivity 5 µS/cm) and pyrogen free.