



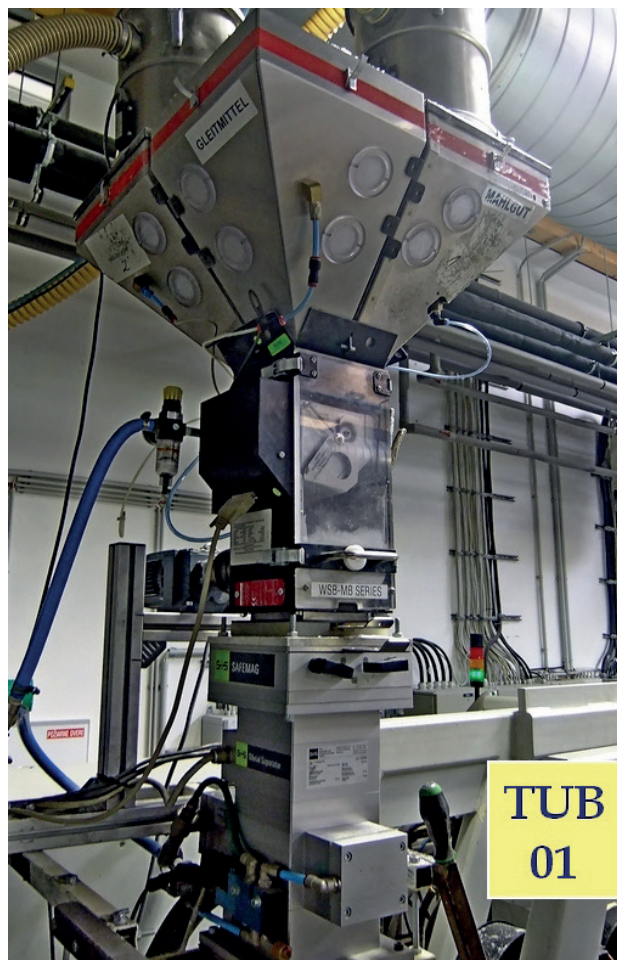
## Quality standard of Minitube's boar semen tube material

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Pig production in the 21st century relies heavily on artificial insemination (A.I.) to ensure efficient breeding programs, fast genetic progress and high biosecurity standards. The benefits of A.I. are based upon the significantly increased availability of superior genetics through the number of semen doses produced from one ejaculate, the storage time of up to one week, and the improved logistics enabling shipment of semen conveniently to any place these genetics are needed.

In order to fully reap these benefits, the boar semen tubes used in the semen production system must be completely suitable for the purpose of semen preservation, transport and insemination. The superior quality of Minitube boar semen tubes is based upon the selection of top grade raw materials, strict QC, complete traceability and state-of-the-art production lines.

Boar semen tubes, or other semen containers like bottles or bags, must be made from materials that do not exert any negative influence on sperm cells during storage and transportation, for example from molecules leaching from the container walls and damaging the semen. Therefore, the materials used to manufacture the containers must be tested and proven to be biologically inactive and sperm friendly. This responsibility lies with the manufacturers of such semen containers. As a specialised and certified producer of semen tubes, Minitube ensures through a rigorous QC system that the quality standard of every single semen tube complies with these requirements.



The material used for boar semen tube production differs profoundly from the material used for semen bags. Bags are made of several layers of plastic and are very flexible. Plasticizers are added to achieve this flexibility; most commonly Phthalate esters which are classified to be potentially teratogenic and detrimental to fecundity. Also the adhesive used to manufacture multilayer plastic bags may contain reprotoxic components. These additives may exert an effect on living sperm cells and organisms, in general. Nerin at al. (2014), reported the finding that compounds from multilayer plastic bags caused severe reproductive failures in inseminated sows. This investigation was based on a case of dramatic increase in the return rate of several sow units which was related to Biphenol A derivatives and other toxic compounds found in the plastic bags used. The toxic compounds were found to migrate into the extended semen, which did not affect the semen quality but led to reprotoxicity during fecundation and/or early embryonic development. The concentration of toxic substances varied within a given batch of bags, thus making an efficient quality control impossible.

Boar semen tubes do not require plasticizers or adhesives for their production. Minitube uses a single high quality plastic raw material for tube production and controls all steps in its proprietary, specialized production facility. Several fully automated production lines manufacture the boar semen tubes by melting high grade plastic granulate.

## Raw material quality

Minitube uses only original and certified raw materials provided by certified suppliers. The granulate material is formulated and manufactured in accordance with the latest ISO and GMP regulations. The raw material batches of Low Density Polyethylene (LDPE) designed for tube production complies with the mandatory Minitube standard of full traceability. Unique batch numbers allow every production lot of boar semen tubes to be completely traced back to every single raw material batch and to all details of production, storage, and other treatment conditions. Audits of the raw material production company are performed annually.

The LDPE used for boar semen production is only produced on production lines which are exclusively designated for LDPE production. The producer is certified for the production of raw material used for manufacturing medical devices.

Also the bags used for packaging the LDPE granulate comply with EC 2023/2006 and provide absolute protection from contamination. They do not contain any recycled material or heavy metals. Each granulate batch is proven to contain no harmful substances such as Arsenic, Cadmium, or Mercury among many others. Biological inactivity is guaranteed.



**Figure 1: Raw material for tube production**

## Raw material compliance with general standards

In addition to the internal Minitube standards, the raw materials used in the production of semen tubes meet all of the following standards:

- EC-Directive 1999/45/EC. LDPE is not a dangerous preparation and is biologically inactive.
- EC Regulation 1907/2006/EC and amendments (referred to as REACH regulation).
- Absence of substances such as those published by ECHA and mentioned in Annex XIV of EC Regulation 1907/206/EC (publication EC Regulation 143/2011 of February 17, 2011).
- Empfehlung III „Polyäthylen“ für Kunststoffe im Lebensmittelverkehr: Empfehlungen des Bundesinstituts für Risikobewertung (BfR) (former BgVV). Status: January 2010. Neufassung der Bedarfsgegenständeverordnung of 23 December 1997, last amendment September 23, 2009, (BGBl I 2009, 3130).
- Commission Regulation (EU) No. 10/2011 of January 14, 2011, effectively replacing EC Commission Directive 2002/72/EC of August 6, 2002, as amended: This material contains no monomers which are regulated with a specific migration limit. This material does not contain
- intentionally incorporated additives which are regulated with a specific migration limit. This material does not contain intentionally
- incorporated dual use additives which are subject to disclosure of adequate information as described in Annex IV of Commission
- Regulation (EU) 10/2011. This material has been manufactured in accordance with the relevant requirements of Commission Regulation EC No. 2023/2006 on good manufacturing practice for materials and articles intended to come into contact with food.
- Sperm tolerance standard as performed with boar semen culture tests and analytical tests established at the Unit of Reproductive Medicine of Clinics, University of Veterinary Medicine Foundation Hannover, a GLP accredited laboratory in accordance with the international norm ISO/IEC 17025.

## Release protocol for each batch of raw material before use in production

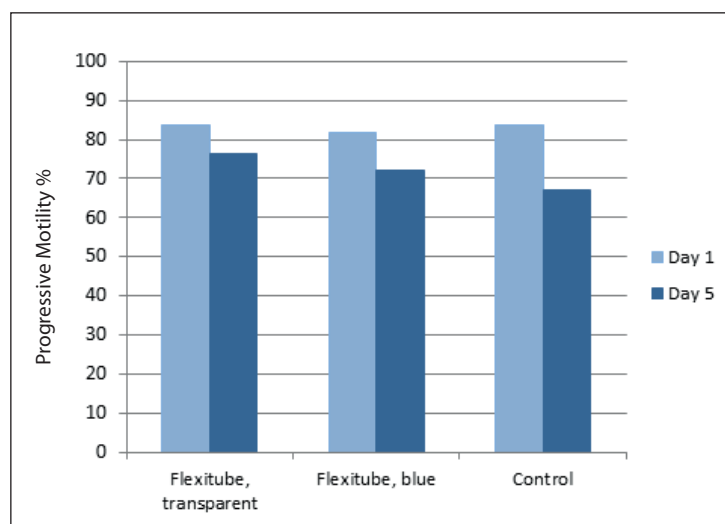
Raw material batch tests are performed on every new batch of raw material Minitube receives. For this test, a sample batch of tubes from the new material is produced and tested with a sperm tolerance examination. Only when all tests of this release protocol are positive can the respective batch of raw material be released for production and be transferred to the production lines of boar semen tubes.

For these tests, boar semen of at least 3 different boars is extended with Androstar Plus, divided into split samples and incubated in the boar semen tubes under test and in glass vials (control). Semen is evaluated for a semen storage period of at least 6 days. All split samples are stored in a semen storage unit at 17°C.

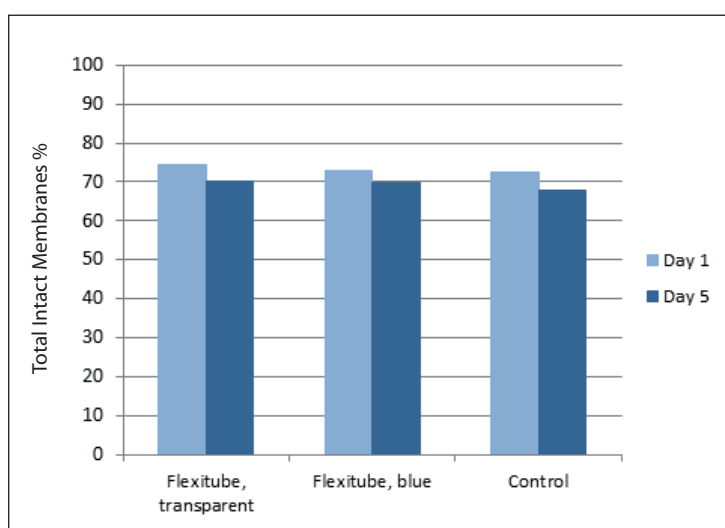
The semen incubated in boar semen tubes made from a new batch of raw material must meet the following criteria in order for the raw material to be released for production:

- Motility readings are at least 90 % of the value of the control sample
- Acrosome integrity is at least 90 % of the value of the control sample
- Membrane integrity is at least 90 % of the value of the control sample

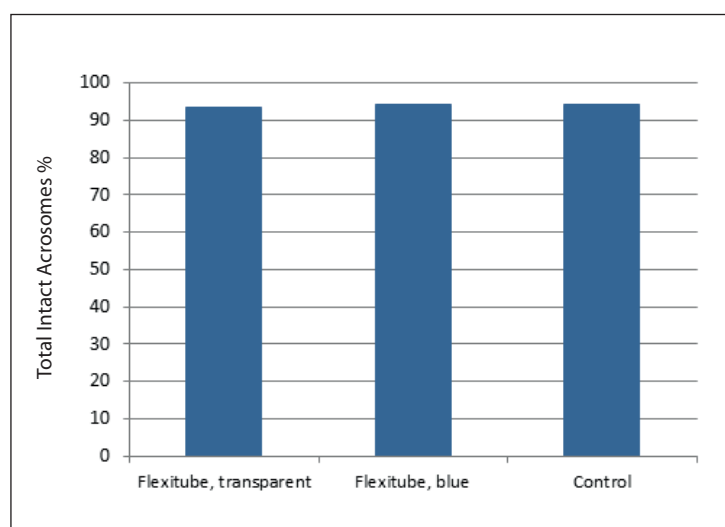
Glass is regarded an inert material which does not emit any substances to the content. Our findings clearly prove the LDPE batches used in Minitube's tube production are perfectly suited for boar semen preservation. In some of our tests, tubes even maintain a motility superior compared to the glass vial.



Graph 1: Progressive motility measured with CASA System



Graph 2: Membrane integrity measured with flow cytometry



Graph 3: Acrosome integrity analysed with 1000 x phase contrast microscopy

Literature: Nerin at al. (2014) „Compounds from multilayer plastic bags cause reproductive failures in artificial insemination“  
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