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## Effect of production management on semen quality during longterm storage in different European boar studs

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#### ABSTRACT

The processing of ejaculates is a fundamental step for the fertilizing capacity of boar spermatozoa. The aim of the present study was to identify factors that affect quality of boar semen doses. The production process during 1 day of semen processing in 26 European boar studs was monitored. In each boar stud, nine to 19 randomly selected ejaculates from 372 Pietrain boars were analyzed for sperm motility, acrosome and plasma membrane integrity, mitochondrial activity and thermo-resistance (TRT). Each ejaculate was monitored for production time and temperature for each step in semen processing using the special programmed software SEQU (version 1.7, Minitüb, Tiefenbach, Germany). The dilution of ejaculates with a short-term extender was completed in one step in 10 AI centers (n = 135 ejaculates), in two steps in 11 AI centers (n = 158 ejaculates) and in three steps in five AI centers (n = 79 ejaculates). Results indicated there was a greater semen quality with one-step isothermal dilution compared with the multistep dilution of AI semen doses (total motility TRT d7: 71.1  $\pm$  19.2%, 64.6  $\pm$  20.0%,  $47.1 \pm 27.1\%$ ; one-step compared with two-step compared with the three-step dilution; P < .05). There was a marked advantage when using the one-step isothermal dilution regarding time management, preservation suitability, stability and stress resistance. One-step dilution caused significant lower holding times of raw ejaculates and reduced the possible risk of making mistakes due to a lower number of processing steps. These results lead to refined recommendations for boar semen processing.

#### 1. Introduction

Consistent high quality semen produced by genetically superior boars is a very important factor in the market position of artificial insemination (AI) organizations (Robinson and Buhr, 2005; Gonzalez-Pena et al., 2014). The risk of mistakes during boar semen processing increases as AI centers become larger and emphasize production time to achieve greater efficiency. Because ejaculate processing has a fundamental influence on the fertilizing capacity of boar spermatozoa, efficiencies gained from size of processing enterprises and associated lesser processing time, must be balanced with the importance of sustaining semen product quality with these conditions.

Currently, extended boar semen intended for AI use is typically stored in 80–100 mL doses for 5 days or less at 16 to 18 °C (Riesenbeck, 2011). Fresh semen is diluted with the aim of extending the longevity of the spermatozoa and in order to increase the use of boars with the greatest genetic value, often to more than 50 semen doses per ejaculate in various European countries.

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Decreasing the number of spermatozoa per AI dose, while simultaneously managing semen in ways to ensure greater quality, are challenges with sperm processing in AI stations (Riesenbeck et al., 2015).

Various exogenous factors could affect the boar sperm cell function during and after processing, for example, the temperature and steps at which semen is diluted (Lopez Rodriguez et al., 2012; Schulze et al., 2013a), the dilution procedure (Schulze et al., 2017) or the storage conditions (Vyt et al., 2007; Schulze et al., 2015). Boar studs should consider the great susceptibility of boar spermatozoa to chilling injury (De Leeuw et al., 1990; Schmid et al., 2013) and dilution shock (Centurion et al., 2003) in order to minimize harmful effects that might compromise fertilizing capacity of the processed semen.

Three different dilution procedures have primarily been adopted by boar studs worldwide, which are the one-, two- and three-step dilution. With the one-step procedure, spermatozoa are diluted isothermally within 30 min after collection to the final volume with the extender being pre-heated to between 30 and 32 °C (Schulze et al., 2013a). With the two-step procedure, semen is initially diluted in a ratio of 1:1 or 1:2 (v:v) at around 32 °C within 30 min, and this is usually followed by a final dilution with an isothermic (32 °C) or hypothermic (21–24 °C) extender within 30 min (Lopez Rodriguez et al., 2012). The three-step dilution is based on a two-step procedure to accelerate the filling process of semen doses which leads to rapid processing with this procedure. With the three-step dilution, half of the final extender volume is added prior to filling (step two) with the remaining extender being dispensed during the dose filling (step three).

The basic problem of identifying critical factors that could be optimized during boar semen processing can be resolved by real-time recording of current production data. At present, no comprehensive information is available regarding time and temperature management in boar studs. One aim of the present study was, therefore, to compare the different semen processing methods in 26 boar studs using software specifically programmed for this purpose. Data were recorded real-time during production, including time periods and temperatures of processing steps during different dilution procedures. Additionally, the effect of production time on boar semen quality was assessed. To accomplish this, sensitive methods were used to detect subtle effects on sperm quality. The second aim was to identify factors related to time and temperature management that affect quality of semen doses after the processing is complete.

#### 2. Materials and methods

#### 2.1. Data acquisition

Field investigations were performed over a 2-year period (2015–2016), excluding the summer months (June to September), in 26 AI boar studs (size 80–470 boars) as part of an external quality control program of the Institute for the Reproduction of Farm Animals (IFN) Schönow. The boar studs were located throughout Germany (n = 23 AI centers) and Austria (n = 3 AI centers). From each of the 26 boar studs, between nine and 19 randomly selected ejaculates from 372 boars (one ejaculate/boar) were analyzed for time (work flow) and temperature management in the semen laboratory during processing and subsequently for semen quality (Fig. 1).

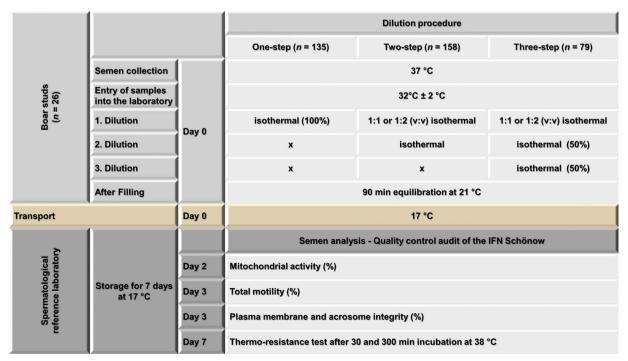


Fig. 1. Schematic illustration of the experimental design.

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