EXTENDED BOAR SEMEN STORAGE ASSOCIATED WITH LOWER SOWS FERTILITY AND LOWER NUMBERS OF PIGLETS BORN ALIVE

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Abstract. The aim of our study was to evaluate fresh extended boar semen quality, stored for four days and used for field sows artificial insemination (AI) and to determine the relationship between semen quality and sow fertility results. Eighty-one ejaculates were collected, assessed for quality, extended, divided into doses and used for AI. In total, 888 crossbreed sows (195 primiparous and 693 multiparous) were serviced twice with the semen of the same boar. At the laboratory, the semen was assessed for sperm concentration, morphology and subjective motility. Sow fertility was evaluated as follows: the non-return rate %, the number of piglets born alive, and the number of stillborns. The lowest non-return rate (72.46 ± 45.00%) was detected in the group with the highest number of pathologic spermatozoa in the semen dose ($P \leq 0.05$). With respect to semen storage, the lowest sow fertility results (non-return rate 73.21 ± 44.48%, lowest number of piglets born alive 10.91 ± 3.31 ($P \geq 0.05$)) and the largest number of stillborn piglets 1.05 ±1.67 ($P \leq 0.05$) were assessed in the group of sows that were inseminated with the semen stored for 72 hours. The correlation between sow's fertility results, semen quality parameters and semen storage day was statistically significant ($P \leq 0.05$).

Keywords: Boar semen quality, sows, fertility rate.

Introduction. Artificial insemination (AI) is the best example of a technology that was rapidly adopted in the swine industry. Swine AI is considered an essential management tool for optimizing production efficiency and maximizing the use of high genetic merit boars. However, a successful AI program depends on a number of factors including, but not limited to, animal health, environment, technical skills, animal management, etc. One important element for AI success is accurate semen evaluation. This evaluation generally provides information on sperm motility, morphology and concentration in the dose (Didion, 2008). It is generally accepted that boar semen with <60% total motility or >20% abnormalities may compromise fertility (Flowers, 1997; Frangež et al., 2005). Little is known about subpopulations of sperm cells within a given semen collection and whether unique motility patterns and specific abnormalities influence boar fertility (Didion, 2008). It has been, however, reported that secondary sperm defects (proximal and distal droplets) reduced the farrowing rate and the litter size in swine (Feitsma et al., 2006).

Today, more than 90% of AI in pig production around the world are carried out with extended liquid boar semen stored chilled for a period of up to 5 days (Riesenbeck, 2011; Schulze et al., 2013). Semen processing involves dilution and cooling of the boar ejaculate to a storage temperature between 15 ºC and 17 ºC. Dilution and temperature management of freshly collected boar ejaculates are the main factors influencing sperm cell function and resistance to hypothermic stress (Schulze et al., 2013). A variety of different extenders are commercially available for use with boar semen. Although short-term extenders have been successful in most European countries, greater distances and diverse production practices have resulted in the use of long-term extenders (Kuster and Althouse, 1999). Studies examining the differences in fertility among long-term extenders have been very limited. Therefore, the aim of this study was to evaluate fresh diluted boar semen quality, stored for four days and used for field AI in sows, and to determine the relationship between semen quality and sow fertility results.

Materials and methods. Ejaculates were collected at a commercial AI centre starting from October 2016 to May 2017, were analyzed for semen quality and used for sow inseminations. Data from 81 ejaculates collected from 41 Landrace boars were used for analyses.

Boar ejaculates were collected on a routine basis at an AI centre with a standardized protocol once per week. The sperm rich fraction was collected using the gloved hand technique. The ejaculate was collected in a prewarmed (38 ºC ± 2 ºC) plastic container. The ejaculate was filtered with a filter to remove gel fraction. Subjective semen motility was assessed under a microscope and the concentration was assessed using a colorimeter. The ejaculate was diluted with Vitasem long-term extender until the final sperm concentration of 2 billion sperm cells per AI dose. After final dilution, polyethylene insemination tubes (Minitüb, GmbH, Tiefenbach, Germany) were filled (80 mL). The tubes were airtight sealed and stored in an acclimatization area (17ºC ± 2ºC). One dose was delivered to the Animal Reproduction Laboratory of the Lithuanian University of Health Sciences, all other doses were transported to a pig farm in an acclimatized transport box (17 ºC ± 2 ºC) and were used for AI on the first, second, third or fourth day. Before use, the doses were kept in a thermostat (17 ºC ± 2 ºC).

In order to evaluate boar semen quality and sow fertility results, 888 crossbreed sows (195 primiparous and 693 multiparous) were selected and inseminated with

fresh semen generally twice per estrus, on the estrus detection day and the next morning, with the same boar's diluted semen. The non-return rate within 60 days of the first insemination (NRR%) and the litter size (total number of piglets born, the number of piglets born alive and stillborns) of primiparous and multiparous farrowings were used as fertility parameters.

Assessment of boar sperm quality parameters

Sperm concentration, morphology and subjective motility of diluted chilled boar semen were assessed using conventional semen evaluation methods (Januškauskas, 2010). Motility of spermatozoa was examined subjectively at 37 °C under phase-contrast microscope Olympus BH2 with a pre-warmed 37 °C stage (Olympus Optical Co., Ltd., Japan) using 400 × magnification. Motility was analyzed on 5-µL aliquots of fresh semen. Motility was analyzed at 0, 24, 48 and 72 hours (day 1, day 2, day 3, day 4) after incubation in the acclimatized box (17 °C ± 2°C) (Friocell, Germany) until analysis. Sperm concentration was assessed in Neubauer improved (Germany) blood cell counting chamber. Sperm tail defects, proximal and distal cytoplasmic droplets, loose heads, acrosome defects, pouch formations, abnormal mid-pieces and incidences of tail abnormalities were determined in wet preparations (an aliquot of semen was fixed in buffered formol-saline solution) under the phase-contrast microscope at 400 × magnification. Sperm head defects (pear shape, narrow at base, abnormal contour, undeveloped, loose abnormal head, narrow, big, little normal, short broad) were determined in dry preparations, stained with SpermBlue (Microptic, Spain). The total number of pathologic spermatozoa were classified as follows: tail defects (sperm tail defects, abnormal mid-pieces and incidences of tail abnormalities); head defects (pear shape, narrow at base, abnormal contour, undeveloped, loose abnormal head, narrow, big, little normal, short broad); and other defects (spermatozoa with proximal loose heads and distal cytoplasmic droplets). The all tested ejaculates were assigned to three groups according to the total number of pathologic spermatozoa in the ejaculate: group A – ≤ 15%, group B – 16–25% and group C – ≤26 %.

Statistical analysis. Statistical analysis was performed using the SPSS statistical package No. 15 for Windows (SPSS for Windows 9.0, SPSS Inc., Chicago, IL, USA). The data included in the model were analyzed using descriptive statistics (mean ± SD) and 1-way ANOVA analysis. The differences between the investigated groups were analyzed by the LSD method (α = 5%). The differences were considered to be statistically significant when: * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$. The correlation between the dependent variables and the strength of the direct relation was evaluated by Pearson correlation coefficients.

Results

Sperm morphology, concentration and sperm motility results at different time are presented in Table 1.

Table 1. **Sperm quality results in dose of fresh diluted boar semen, used for AI**

Parameters (n = 81)	**Mean ± SD**	**Min - Max**
Sperm motility_0 hour, %	66.99 ± 11.43	45–85
Sperm motility_24 hours, %	65.12 ± 16.18	25–85
Sperm motility_48 hours, %	56.48 ± 11.57	30–70
Sperm motility_72 hours, %	56.37 ± 13.00	15–70
Concentration billion / dose	3.18 ± 0.68	1.9–6.1
Sperm head pathologies, %	1.09 ± 0.84	0–4.5
Sperm tail pathologies, %	5.65 ± 6.71	0–31
Other pathologies, %	4.10 ± 3.71	0–21.5
Overall pathologies, %	10.85 ± 8.84	1.5–39

In group A with the smallest number of pathologic spermatozoa, the average of sperm motility was 59.25 ± 10.24%. In group C with the largest number of pathologic spermatozoa sperm motility was 29.25% lower ($P \leq 0.05$). The same result is reflected in the sow fertility results. Although the higher non-return rate was detected in the boar group with the average semen quality. The lowest non-return rate (72.46 ± 45.00%) was detected in group C with the highest number of total pathologic spermatozoa in the semen dose ($P \leq 0.05$) (Fig. 1).

Sow fertility results (non-return rate and number of piglets born alive and piglets born dead in a farrow) correlated with semen quality parameters and semen storage day (Table 2).

The highest percentage of the non-return rate (81.48 ± 39.58%) and the number of piglets born alive (11.85 ± 3.44) were in sows which were inseminated with the semen stored 48 hours (N = 112). The number of piglets born alive and the percentage of the non-return rate in sows inseminated with first and second day stored semen were almost the same: piglets born alive 11.19 ± 3.29on the first day (N=267) and 11.37 ± 3.34 on the second (N=340) day of semen storage non-return rate 81.16 ± 39.17% and 80.95 ± 39.31%, respectively, ($P \geq 0.05$). The lowest sow fertility results were obtained when sows were inseminated with the semen stored for 72 hours (insemination day 4, N = 27): non-return rate 73.21 ± 44.48 % and number of piglets born alive 10.91 ± 3.31 ($P \geq 0.05$) (Fig. 2). In this group, the largest number of piglets born dead was detected (1.05 ± 1.67; $P \leq 0.05$).

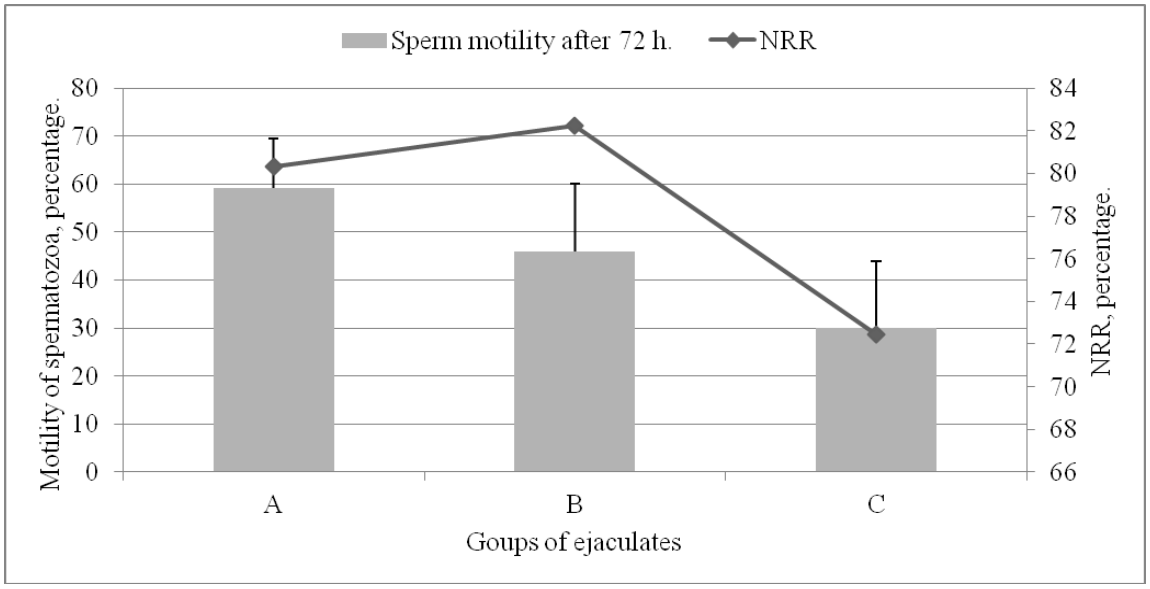

Figure 1. **Sperm motility after 72 hours and non–return rate results in three groups of ejaculates**

Table 2. **Correlation coefficients (R) between sperm quality parameters and sow fertility data**

Parameters	**NRR, %**	**Piglets born alive**	**Piglets born dead**
Semen stored day	−0.039	0.036	0.083*
Sperm motility_0 hour, %	0.066	0.054	0.042
Sperm motility_24 hours, %	−0.046	0.130*	0.029
Sperm motility_48 hours, %	−0.020	0.030	0.026
Sperm motility_72 hours, %	0.115**	0.052	0.007
Concentration billion / dose	0.031	−0.016	0.053
Sperm head pathologies, %	−0.101**	−0.016	0.053
Sperm tail pathologies, %	−0.002	0.031	0.001
Other pathologies, %	−0.043	−0.023	0.072
Overall pathologies, %	−0.029	−0.021	0.041*
* P < 0.05; ** P < 0.01; and *** P < 0.001			

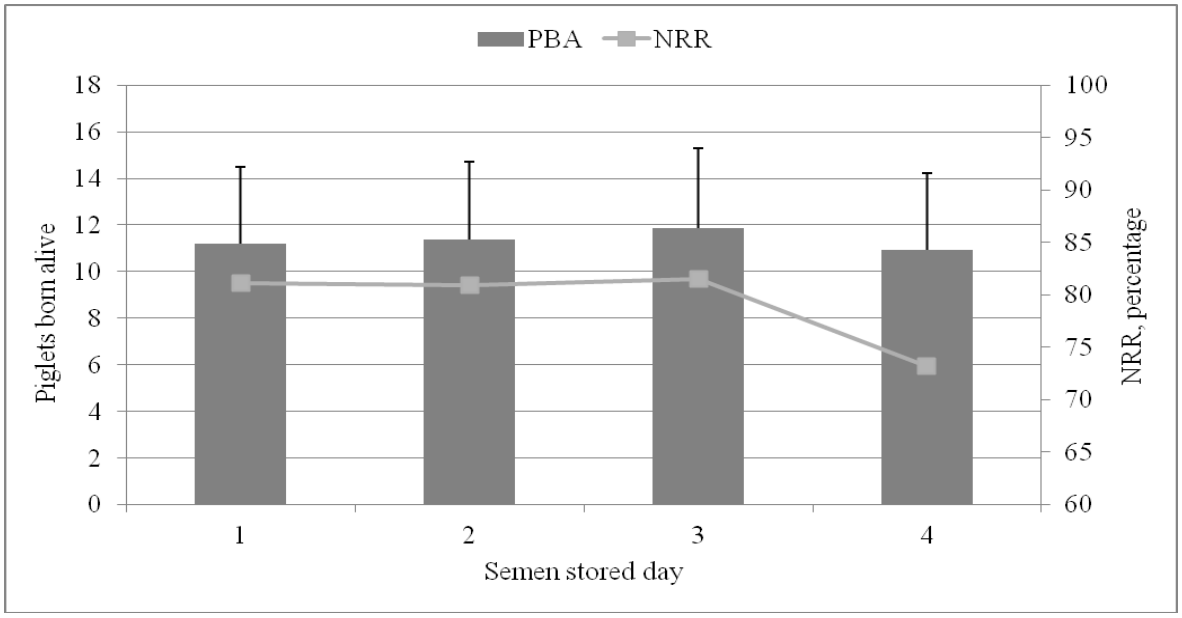

Figure 2. **Number of piglets born alive (PBA) and percentage non–return rate results on different semen storage days**

The results of our study showed that the age of sows also had a significant effect on the number of piglets born alive and dead per farrow. The percentage of the non-return rate in multiparous sows was 81.1 ± 39.18%, and in primiparous sows it was 76.41 ± 42.57% ($P \geq 0.05$). The average number piglets born alive in multiparous sows was by 1.56 ± 0.38 higher than in primiparous sows ($P \leq 0.05$). The number of piglets born dead in primiparous sows was by 0.29 ± 0.01 lower than in multiparous sows ($P \leq 0.05$) (Fig. 3.).

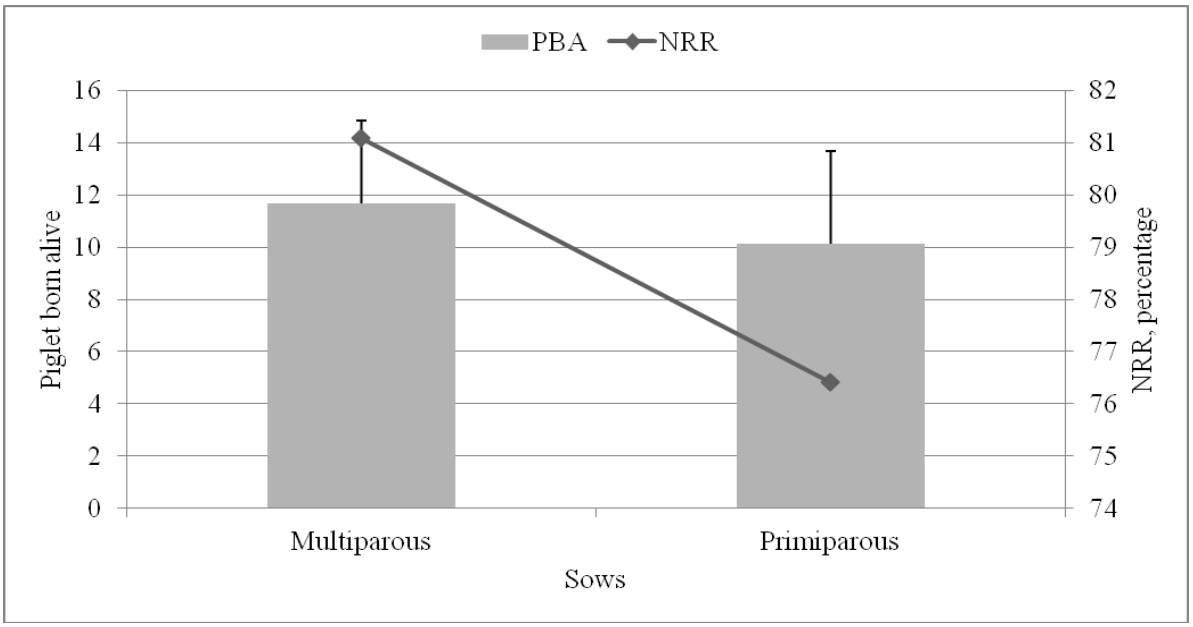

Figure 3. **Number of piglets born alive and non–return rate results in primiparous and multiparous sows**

Disccusion. It is generally accepted that there is a large variation in fertility results, mainly caused by farm and sow-related parameters (Hanenberg et al., 2001). Factors that affect this variation in fertility can be minimized by breeding, management, and also by artificial insemination. Remaining variation in pig fertility is explained by boar- and semen-related parameters. Nowadays, AI is a tool for efficient distribution of high quality genetics and efficient running of a genetic program. AI centers should always aim to minimize their effect on variation in pig fertility and at the same time know which role they play in the pig-breeding program by, among others, semen quality assessment. It is essential for AI centers to guarantee that only high quality ejaculates will be processed further.

Sperm motility is commonly believed to be one of the most important semen quality characteristics (Ruiz-Sánchez, 2006). In this retrospective study, fresh sperm motility is used as an example parameter which possibly affects variation in pig fertility. The usefulness of semen motility assessments to judge semen quality has been debated although it is commonly used and has been shown to correlate with the fertilizing capacity of semen (Holt 1997; Flowers, 1997; Tardif et al., 1999; Gadea, 2005). Besides sperm motility, there are several other boar- and semen-related parameters affecting pig field fertility, such as the number and the quality of spermatozoa inseminated (Tardif et al., 1999; Holt, 1997).

This study was designed to evaluate the quality of fresh boar semen diluted with long–term diluent, stored for four days and used for artificial insemination of field sows. Subjective sperm motility, morphology and concentration in the dose for AI and the relationship between semen quality parameters and sow fertility results were analyzed. The results showed the correlation between sperm motility and the overall sperm pathologies. The non-return rate correlated with sperm motility after 72 hours ($P < 0.01$) and negatively correlated with sperm head pathologies ($P < 0.01$). Although previous studies showed that semen storage did not influence the farrowing rate or the litter size (Rozeboom et al., 2000; Broekhuijse et al., 2012), our results showed the influence of semen stored day on the non-return rate and the total number piglets born dead in a farrow ($P < 0.05$). Sperm motility is only one of several factors that affect the fertilization process. It has been demonstrated that when motility is 60% or higher there is no relationship between in vivo and in vitro estimates of fertility (Kuster, 1999). The present results support this finding. The lowest sow fertility results were estimated when sows were inseminated with the semen stored for 72 hours ($P \geq 0.05$), with the sperm dose where the average subjective sperm motility was 56.37 ± 13.00%.

The recommended storage period of liquid boar semen depends on many other factors than the semen extender such as temperature (Johnson et al., 2000; Zou and Yang, 2000; Schulze, 2013), the number of spermatozoa in AI doses (Johnson et al., 1988), semen quality (Weitze, 1991), and the time between ovulation and AI (Haugan, 2007), which could result in the effect of semen ageing on fertilizing capacity being more pronounced in gilts than in sows (Anil et al., 2004).

Motility is very important for semen quality. However, motility alone does not secure fertilizing capacity. Normal cell morphology also need for fertilization process. The results from the trial indicated the average optimum pathologic cells in the dose, although the ranges of pathologic spermatozoa vary a lot. This investigation demonstrates that semen quality was gradually reduced during 72 hours of storage, a result which is in line with other investigations (Kommisrud et al., 2002). There was a small but significant reduction in motility at the end of the experimental period, the percentage of motile spermatozoa was maintained at a quite high level event after four days storage which corresponds to findings in other investigation (Schulze, 2013). Didion (2008) showed no significant relation for any unique motion parameter with fertility data, which is in contrast to the other studies (Holt et al., 1997; Vyt et al., 2008). It remains unknown, therefore, whether the relatively small albeit significant difference in semen quality affect differences in fertility.

It is, however, a fact that there in considerable variation among boars concerning the fertilizing capacity of semen during storage. On the other hand, there are several other factors which might influence fertility of stored semen. Individual variation concerning the chemical composition of the ejaculate as well as the number of morphologically abnormal spermatozoa and seminal plasma might be of importance (Kommisrud et al., 2002).

To conclude, semen storage day and sperm quality are associated with lower sow fertility and the number of piglets born alive. Only fresh diluted boar semen with subjective motility of 60% or higher and less than 25% of morphological subnormalities can be stored and used for AI 4–5 days without compromising sow fertility results.

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