EVALUATION OF THE QUALITY OF BOAR SEMEN DILUTED IN VARIOUS EXTENDERS USING A LONG-TERM THERMORESISTANCE SURVIVAL TEST

Frydrychova S., Lustykova A., Seifert J., Kucharova S., Falk Lipensky J. and Rozkot M.

Institute of Animal Science, Prague Uhříněves, workplace Kostelec nad Orlicí, Czech Republic

Abstract

The aim of this study was to determine the quality of boar semen in samples prepared by dilution in long-term commercial extenders using a long-term thermoresistance survival test. Over the period of one year, sixteen ejaculates were collected from eight Přeštice black-pied boars from one AI centre. Basic semen quality parameters were determined. The semen was diluted in Androstar plus, Androstar premium and Spermax extender at a dilution ratio of 1+2 and the prepared samples were stored at 17°C. The test was performed after storage for up to 168h and sperm motility was evaluated during incubation at 38°C in water bath after the 1st, 3rd and 5th hour. During storage and incubation, no significant differences in sperm motility were observed between the extenders ($p>0.05$). Sperm motility was statistically significantly reduced in all tested extenders after 96 and 168h of storage ($p<0.05$). Negative correlation was observed between sperm motility and decreasing pH ($p<0.05$). The best temperature stability was found in the Androstar premium extender where a reduction in sperm motility was observed in the 5th of incubation, while in the Androstar plus and Spermax extender, a reduction was observed in the 3rd hour ($p<0.05$). In conclusion, this study did not find differences in sperm motility between the tested extenders. The best temperature stability was found in the Androstar premium extender compared to the others. According to the results these tested extenders are suitable for the production of insemination doses and in the process of sperm cryopreservation.

Key Words: Boar semen quality, extenders, long-term thermoresistance survival test

Artificial insemination has become the most used biotechnological method in pig reproduction (Bortolozzo et al., 2015). Boar semen extenders for maintaining sperm viability, quality and fertilization capacity can be divided into short-term, medium-term and long-term extenders (Feugang et al., 2019). Long-term extenders are used to provide additional protection for spermatozoa during storage (thanks to improved buffering agents, antioxidants, antibiotics etc.) and have an impact on transport of insemination doses to consumers (Waberski et al., 2019; Knox, 2016; Sangma et al. 2020) and also the choice of extender for pre-dilution rate during cryopreservation can lead to an increase in semen quality after thawing (Kaeoket et al., 2011). The sperm thermal resistance test is a laboratory test used to evaluate the viability and metabolic stability of sperm exposed to a temperature of 38° C simulating the physiological conditions to which sperm are exposed in the female reproductive tract after insemination (Schulze et al., 2019). The parameters of the TRT test of boar semen can be used to explain differences in pig fertility (Schulze et al., 2013).

Přeštice black-pied pig is an original national breed in the Czech Republic and has been included in the program of preservation of Animal Genetic Resources.

The aim of this study was to determine the quality of boar semen in samples prepared by dilution in various long-term commercial extenders using long-term thermoresistance survival test for the later preparation of insemination doses or for cryopreservation.

Material and Methods

During one year, sixteen ejaculates were collected from eight fertile AI boars of Přeštice black-pied pig aged 1.5 to 5 years. The study was conducted at the main insemination station for Preštice black-pied boars in the Czech Republic. The boars were kept in the same housing, feeding and breeding conditions.

Ejaculates were collected using the gloved-hand technique and the gel portion was removed by using double gauze. The following semen quality parameters were evaluated in the fresh native boar semen: sperm volume, sperm motility, sperm concentration, percentage of live spermatozoa, proportion of morphologically abnormal spermatozoa (MAS) and pH. Sperm concentration was measured with IMV AccuRead (manufactured in the USA for Biochrom Ltd. Cambridge UK). MAS were assessed according to the staining method of Čeřovský (1976) and two hundred spermatozoa per slide were evaluated microscopically under oil immersion and 1500× magnification. Sperm motility was subjectively assessed by microscopic estimation of the number of spermatozoa moving in the field of view of a phase-contrast microscope with a heated stage (38 °C) at 200× magnification. Each sample was examined in three different microscopic fields and motility was expressed as the percentage of spermatozoa exhibiting normal progressive forward movement. Percentage of live spermatozoa was estimated by supravital staining technique using the eosin-nigrosin stain mixture (Věžník et al., 2004). One drop from each sample was mixed with 1 drop of 1% eosin Y, then 2 drops of 10% nigrosine were added after 30s. Two hundred spermatozoa per slide were evaluated under a light microscope (1500×). The pH was assessed using the Hanna precision pH meter at 20°C (Sigma-Aldrich, Czech Republic) and osmolality extenders with the Marcel Osmometr OS 300 (2THETA ASE, Czech Republic). The long-term thermoresistance survival test (TRT) was assessed in diluted boar semen. The semen was extended in dilution rate 1+2 in long-term extenders Androstar plus, Androstar premium (Minitube, Germany) and Spermax (Magapor, Spain) and prepared samples were stored 17°C up to 168h. The test was performed on 3 ml samples kept at 38°C in water bath after storage time 24h, 48h, 72h, 96h and 168h and motility of spermatozoa was evaluated in the 1st, 3rd and 5th hour during the incubation.

Basic statistical characteristics of the results – arithmetic means, standard deviations, standard error of the mean (SEM), correlation coefficients and significance were calculated by the QC Expert program (TriloBite Statistical Software s.r.o., Pardubice, Czech Republic). All data were tested for normality before analysis. The data were analysed by statistical analysis of variance ANOVA followed by the Fisher test ($p<0.05$).

Results and Discussion

The mean values of the initial quality of boar semen was as follows: sperm motility 86.88±2.59%, sperm concentration 420.13±114.04×10^3/mm^3, MAS 22.5.0±11.87%, sperm volume 308.75±46.43 ml, percentage of live spermatozoa 78.06±6.58% and pH 7.55±0.17. Table 1 shows the mean values and standard deviation of osmolality and pH of tested extenders and diluted samples after 24 and 168h of storage. Statistically significant differences ($p<0.05$) were found in the osmolality of the tested extenders. Androstar plus extender recorded a higher value than Androstar premium and Spermax extenders but according to the results, this had no effect on sperm survival. Yeste et al. (2010) concluded that osmotic resistance in sperm viability, sperm morphology and acrosome-intactness in the above-mentioned treatments could be assessed along with classical parameters to better predict the fertilising ability of a given ejaculate. The osmolality values of the extenders corresponded to the optimal condition for boar sperm in the range 289 to 327 mOsmol/kg (Schilling and Vengust, 1986). No statistically significant differences in pH values were found between diluents, but a negative correlation (r=-0.45, p<0.05) was observed between sperm motility and slowly decreasing pH. The diluents maintained a stable pH, which is essential for optimal sperm motility and longevity. Vyt et al. (2004) pointed out in their study that pH usually increases by 0.3–0.5 units during the first days of storage and this increase significantly correlates with reduced motility, especially in long-term extenders.

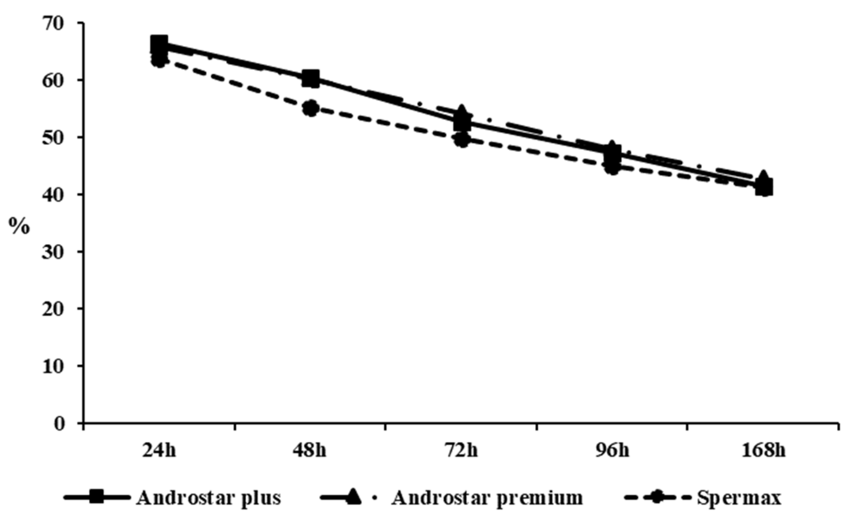
Progressive sperm motility is an indicator of good metabolism and membrane integrity of sperm (Johnson et al., 2000). Our obtained mean values of sperm motility in the tested extenders during long-term TRT are presented in Figure 1. No significant differences in sperm motility were observed between the extenders (p>0.05). Sperm motility was statistically significantly reduced in all tested extenders after 96 and 168h of storage (p<0.05). Teixeira et al. (2015) and Kaeoket et al. (2010) also found in their study that sperm motility was not significantly affected by the type of extender during the test period. In contrast, Sangma et al. (2020) observed differences in progressive sperm motility between extenders during storage time.

Table 1. Mean values and standard deviation of osmolality and pH

Item	Osmolality (mOsmol/kg)	pH extender	pH sample 24h	pH sample 168h
Androstar plus	329.00±7.11[a]	7.30±0.03	7.46±0.18	7.42±0.55
Androstar premium	304.25±4.03[b]	7.13±0.60	7.43±0.22	7.33±0.53
Spermax	298.38±16.35[b]	7.42±0.15	7.66±0.24	7.65±0.55

[a,b] means within the column [a,b] p<0.05

Figure 1. Sperm motility (%) in different extenders during long-term TRT



The percentage of sperm motility evaluated during the incubation period was best in the 1st in the Androstar plus extender and then in the 3rd hour in the Androstar premium extender. No significant differences in sperm motility during the incubation period were observed between the extenders ($p>0.05$). The best temperature stability was found in the Androstar premium extender, as a significant decrease in sperm motility was observed only at the 5th hour of incubation, while in the Androstar plus and Spermax extenders the decrease was observed already in the 3rd hour ($p<0.05$). The following changes are reported in Figure 2. Stankovieć et al. (2012) documented that thermal resistance test revealed significant changes of progressive motility rate of spermatozoa after exposure to controlled heat stress. Jakob et al. (2019) also reported that the proportions of motile and progressively motile sperm decreased with storage time and in the course of TRT incubation time. High temperatures and/or long periods of incubation during TRT also negatively affects bovine sperm motility (Vianna et al., 2009).

Figure 3 presented the comparison of total mean values of sperm motility between tested extenders and their influence on sperm survival during long-term TRT. There were found no statistically significant differences between extenders. The best sperm motility was in the Androstar premium 54.2% then Androstar plus 53.68% and Spermax 50.96%. Waterhouse et al. (2004) noted that long-term sperm extenders not only prolong sperm viability but also better preserve the structural integrity of the plasma membrane and acrosome compared to short-term extenders.

Figure 2. Mean values of sperm motility in the 1st, 3rd and 5th hour during the incubation

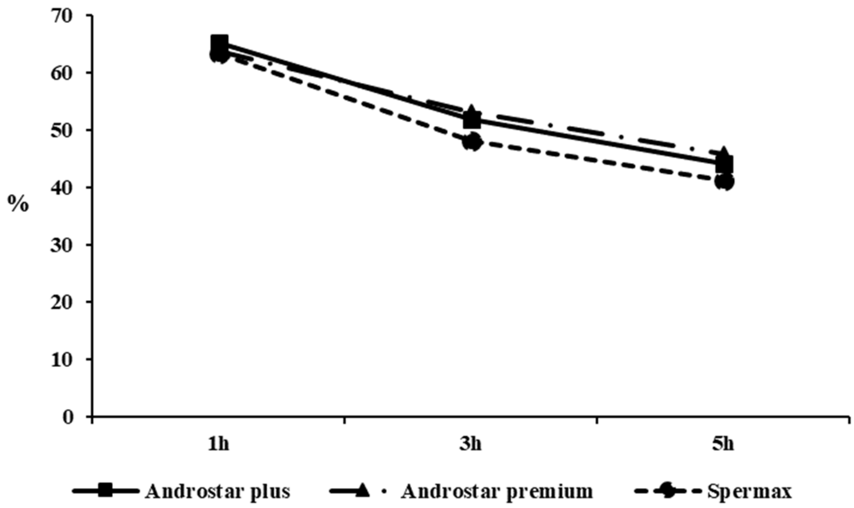
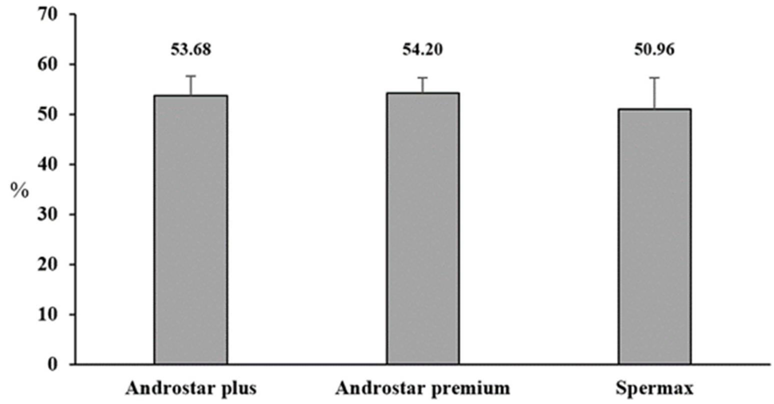


Figure 3. Comparison of total values of sperm motility between tested extenders (mean±SEM)



Conclusion

In conclusion, this study did not find statistically significant differences in sperm motility between the tested long-term extenders when using the long-term TRT test. Sperm motility was statistically significantly reduced in all tested diluents during storage and incubation. Negative correlation was observed between sperm motility and decreasing pH. The best temperature stability was found in the Androstar premium extender compared to other extenders. Androstar plus extender had a higher osmolality value, but according to the results, this had no effect on sperm survival. According to the results these tested extenders are suitable for the production of insemination doses and in the process of sperm cryopreservation.

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Corresponding Address:
Ing. Soňa Frydrychová, Ph.D.
Institute of Animal Science Prague
Department of Pig Breeding Kostelec nad Orlicí
Komenského 1239, 51741 Kostelec nad Orlicí
Czech Republic
E-mail: frydrychova.sona@vuzv.cz

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