INFLUENCE OF DIFFERENT EXTENDERS, DILUTION RATE AND STORAGE TIME ON BOAR SPERM PROGRESSIVE MOTILITY

Lipenský J., Lustyková A., Frydrychová S., Rozkot M., Václavková E.

Institute of Animal Science, Prague, Czech Republic

Abstract

The aim of this study was to evaluated the preservation ability of three commercial extenders frequently used in the Czech Republic and evaluated the effect of dilution rate and storage time on boar sperm progressive motility. The semen was extended in a commercial boar semen extender Androhep Plus, BTS and VIP3 and was stored at a temperature 17°C up to 96 hours. Sperm motility was evaluated each day at the 1st, 3rd and 5th hour during the incubation in water bath. Sperm progressive motility was significantly decreased for all extenders with storage period ($p < 0.001$) and the dilution rate had the significant effect on all tested extenders. The best sperm survival ability was observed in extender Androhep Plus. In conclusion, different sperm survival ability based on used extender, dilution rate and storage time was noted during this study.

Key Words: Extender, dilution rate, storage time, sperm

Artificial Insemination (AI) plays a fundamental role in pig reproduction in the Czech Republic, therefore the quality of insemination doses, mostly produced at AI centers, can affected the economy of pig farms. The goal of AI centers is the highest quality at the lowest cost as possible. Due to low price and frequency of boar semen collection on AI centers, short-term extenders are widely used. There are numerous commercial extenders for preservation of boar semen. As the previous studies presented, they differ not only in price but also in their ability to maintain sperm motility in time (Vyt et al. 2004, Frydrychová 2010, Lustyková 2010). Semen motility is been considered as an important characteristic for fertilizing capacity of semen cells (Feitsma 2009). Estimation of motility has fundamental importance in the daily quality control of the semen. The percentage of motile spermatozoa is used to calculate the required degree of dilution and to estimate the number of "intact" spermatozoa per insemination dose (Johnson et al. 2000). Along the other factors, dilution rate can affected sperm motility (Marcos et al. 1991, Maxwell and Johnson 1999, Hrstková et al. 2001) and play an important role in AI doses production. The aim of this study was to evaluated the preservation ability of three commercial extenders frequently used in the Czech Republic and evaluated the effect of dilution rate and storage time on boar sperm progressive motility.

Material and Methods

Seven ejaculates from three fertile boars of Přeštice black-pied pigs and four hybrid boars were used in this study. Ejaculates were collected by a gloved-hand technique and the gel portion was removed by using double gauze. The following semen quality parameters were evaluated: sperm motility, sperm concentration, morphologically abnormal spermatozoa and pH value after storage (24h and 72h). Sperm progressive motility was evaluated subjectively by microscopic estimation of the number of sperm moving in a visual field of phase contrast microscopy with a heating stage (38°C) at 100x magnification. Each sample was examined at three different microscopic fields and motility was expressed as percentage of sperm showing normal forward progressive movements. Sperm concentration was estimated using a Bürker counting chamber. Morphologically abnormal spermatozoa were evaluated according to the staining method of Čeřovský (1976) and evaluated microscopically under oil immersion and 1500x magnification. The long-term thermo-resistance survival test was assessed in diluted boar semen. The semen was extended (dilution rate 1+4, 1+8, 1+16) in a commercial boar semen extender Androhep Plus, Beltsville Thawing Solution (BTS) and VIP3 and was stored at a temperature 17°C up to 96 hours. Beltsville extender and VIP3 extender are classified as short-term extenders. The test was performed on 3 ml samples kept at 38°C in water bath after storage time 24h, 48h, 72h and 96h and motility of spermatozoa was evaluated at the 1st, 3rd and 5th hour during the incubation. The thermo-resistance stability was calculated as 3rd or 5th hour motility value / 1st hour motility value * 100.

Statistical characteristics of the results were calculated using the QC Expert statistical program. Statistical significance was checked by the analysis of variance ANOVA and t-test at significance levels of $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

The initial quality of semen used in this study is presented in Table 1. The mean value of tested parameters was as follows: native sperm motility 75.71 %, sperm concentration 418.29 $10^3/mm^3$, morphologically abnormal spermatozoa 19.7 %. The mean motility of extended semen decreased to 60.6 % after 2 hours whereas only minimal non-significant differences between tested extenders were found.

Figure 1 shows the effects of extenders and their influence on boar spermatozoa during long-term thermo-resistance survival test. The extender Androhep Plus was not outdone by the other tested extenders in this test ($p<0.001$). Comparison of mean values for motile spermatozoa in extenders and storage periods during long-term thermo-resistance survival test shows Figure 2. Sperm progressive motility was significantly decreased for all extenders with storage period ($p<0.001$) however no significant differences between 48 h and 72 h values were found. The

highest significant decrease was observed in BTS extender whereas the lowest motility decrease was found in Androhep Plus extender.

The highest percentage of sperm progressive motility evaluated at the 1st, 3rd and 5th hour during long-term thermo-resistance survival test was found in extender Androhep Plus. This extender also achieved the best thermo-resistance stability in this test (Figure 3 and 4). Low thermo-resistance stability was noted in BTS extender when progressive sperm motility evaluated after 5th hour drop below 50 % of its 1st hour value ($p < 0.001$).

The effect of dilution rate on sperm motility in all tested extenders shows Figure 5. The dilution rate had the significant effect on all tested extenders especially on extender VIP3. The best dilution stability was observed in extender Androhep Plus. The semen samples extended at dilution rate 1+16 maintained very low sperm progressive motility after 24 hours in BTS extender and after 48 hours in VIP3 extender. Insufficient results were also observed in semen samples extended in BTS and VIP3 extenders at dilution rate 1+8.

The highest pH values were observed in samples diluted by BTS extender and were significantly higher in 1+16 dilution rate than values observed in Androhep Plus and VIP3 extenders. The results for pH values are shown in Table 2.

Figure 1. Comparison of preservation effects of BTS and VIP3 extenders to Androhep Plus (100%) during the long-term thermo-resistance survival test

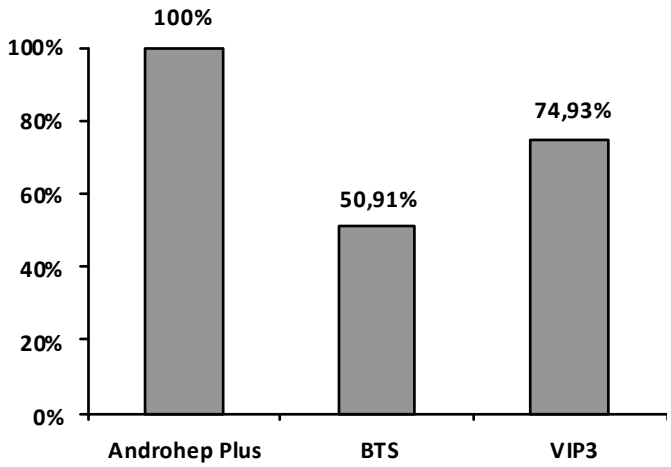

Figure 2. Comparison of mean values for sperm progressive motility (%) in different extenders during the long-term thermo-resistance survival test

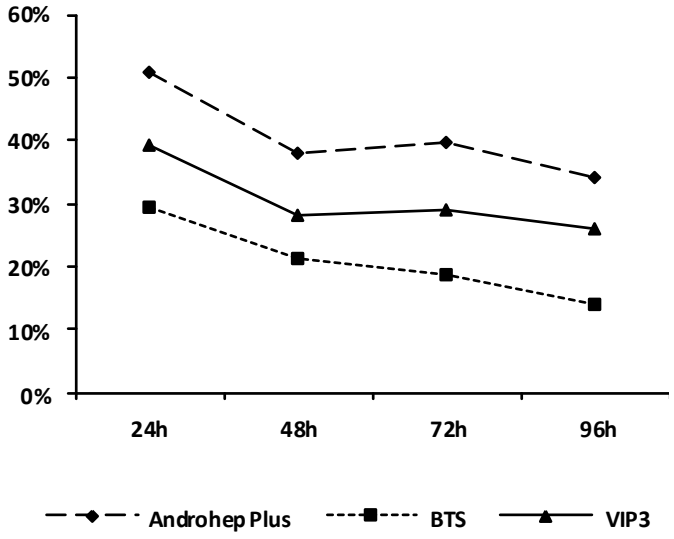

Figure 3. The average thermo-resistance stability evaluated after 3rd hour during the incubation (1st hour = 100%)

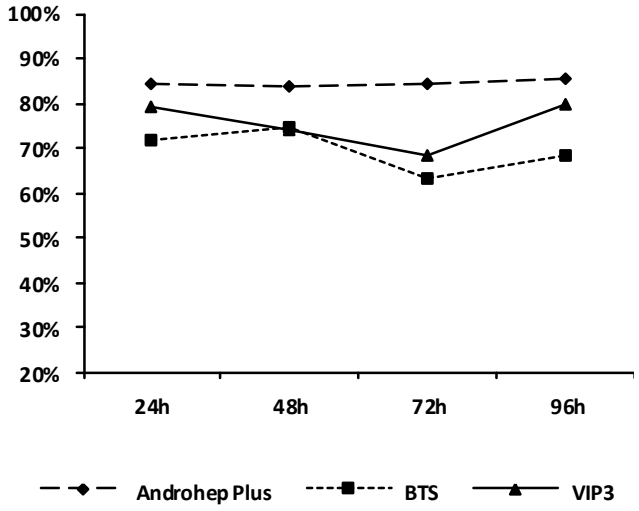

Figure 4. The average thermo-resistance stability evaluated after 5th hour during the incubation (1st hour = 100%)

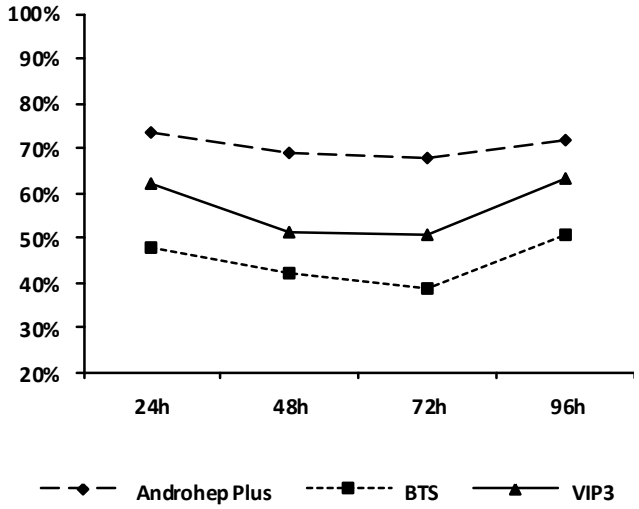

Figure 5. The effect of dilution ratio on sperm progressive motility evaluated 1st hour during the long-term thermo -resistance survival test (mean ± SEM)

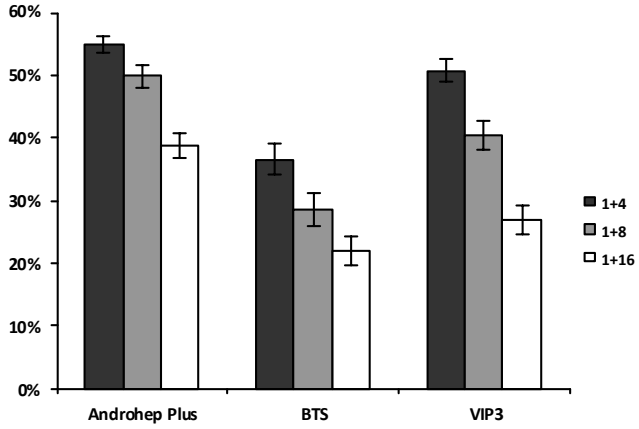

Table 1. The initial quality of semen used in this study

Boar No	Sperm concentration (10^3/mm^3)	Morphologically abnormal spermatozoa (%)	Sperm progressive motility after collection (%)	Sperm progressive motility 2 hours after dilution (%)		
				Androhep Plus	BTS	VIP3
1	610	24.0	80	63	72	73
2	415	18.5	80	60	53	63
3	834	13.5	90	75	70	70
4	218	50.0	55	35	33	30
5	218	14.0	80	63	70	68
6	309	10.5	75	67	67	67
7	324	7.5	70	63	57	53

Table 2. pH values after 24 and 72 hours (mean ± SEM)

Dilution rate	Androhep Plus		BTS		VIP3	
	24h	72h	24h	72h	24h	72h
1+4	7.21 ± 0.05^{a}	7.15 ± 0.08^{a}	7.39 ± 0.07^{a}	7.38 ± 0.12^{a}	7.21 ± 0.07	7.23 ± 0.16
1+8	7.32 ± 0.04	7.29 ± 0.04^{a}	7.56 ± 0.06^{a}	7.58 ± 0.08^{aA}	7.25 ± 0.05	7.17 ± 0.10^{B}
1+16	7.38 ± 0.03^{bC}	7.40 ± 0.02^{bE}	7.75 ± 0.05^{bcA}	7.79 ± 0.03^{bD}	7.34 ± 0.05^{C}	7.35 ± 0.06^{E}

^{a,b,c} means within the column ^{a,b} $p < 0.05$; ^{a,c} $p < 0.01$
^{A,B,C,D,E} means within the row ^{A,B} $p < 0.01$; ^{A,C} and ^{D,E} $p < 0.001$

Discussion

The VIP3 extender was significantly better than BTS extender in this survival test while high-priced extender Androhep Plus was the best of the tested extenders. The difference between tested short-term extenders and Androhep Plus extender, furthermore, increased with depending on the dilution rate in VIP3 extender and depending on the dilution rate and storage time in BTS extender. Dubé et al. (2004) also found the evident difference in sperm motility when compared Androhep Plus and BTS extender and concluded that Androhep Plus was significantly superior to BTS extender. Corcini et al. (2011) noted that sperm motility declined from 85% to nearly 30% during five days storage in BTS extender.

The highest dilution stability was observed in Androhep Plus extender. In general, sperm progressive motility was negatively affected by storage time and by dilution rate. However, the negative effect of dilution rate and storage time depended on the used extender. In addition, an important influence of boar individuality on sperm motility was noted in this study. This influence was especially high in BTS extender. Kommisrud et al. (2002) reported influence of boar as factor that might have a significant influence on sperm motility and acrosome integrity in BTS extender and according to Estiene et al. (2007) short-term extenders can be successful at maintaining motility up to seven days after collection. However, single inseminations of semen stored in BTS up to 62 hours negatively affected litter size (Haugan et al., 2005). Stančić et al. (2012) suggested determining the adequate dilution rate and storage time for each ejaculate, while taking into account the spermatozoa concentration in the native semen.

The data shown that thermo-resistance stability tested in water bath was slightly affected by storage time but evident effect of tested extenders, dilution rate and incubation time was noted. Low thermo-resistance stability was noted in BTS extender while the highest thermo-resistance stability was found in Androhep Plus extender. Lustyková et al. (2010) also obtained similar results of the thermo-resistance survival ability of Androhep extender.

In this study pH mean value appeared to be stable in time in all tested extenders. No significant difference was found between 24h and 72h mean values. However, the pH value tended to increase or decrease depending on the influence of boar. Furthermore, higher pH values were found in higher dilution rates. Kaeoket et al. (2010) reported that the pH values of the extended semen decreased during storage time while according to Vyt et al. (2004) pH increased by 0.3–0.5 in the first days of storage and was significantly correlated with a decrease in motility. Described pH-rise could be explained by the presence of a bicarbonate buffering system in the extender and by the amount of air present within the sperm-containing tube (Vyt et al., 2007).

Conclusion

The Androhep Plus was the best of the tested extenders. Different sperm survival ability based on used extender, dilution rate, storage time and influence of boar was noted during this study. The results indicated that it is important to control sperm motility (AI doses quality) before dilution and after dilution as well as during whole storage period.

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Corresponding Address:
Ing. Jan Lipenský
Institute of Animal Science Prague
Department of Pig Breeding
Komenskeho 1239
51741 Kostelec nad Orlicí
Czech Republic
E-mail: lipensky.jan@vuzv.cz

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