EFFECTS OF LONG-TERM COMMERCIAL EXTENDERS FOR LIQUID STORAGE OF BOAR SEMEN

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Abstract

The objective of this study was to compare preservation ability of five long-term commercial semen extenders by means of spermatozoa long-term thermo-resistance survival test. Ejaculates from 21 fertile boars were collected by hand method. Semen gel free volume, motility, viability, sperm concentration, total morphologically abnormal spermatozoa and total number of spermatozoa per ejaculate were determined. The samples of diluted sperm in a semen-dilution rate of 1+4 in Androhep (A), Androstar (AS), Androstar plus (AS^+), LD and M III were stored at a temperature 17°C up to 96 h. The test was performed on 3 ml samples kept at 38°C in water bath each day and motility of spermatozoa was evaluated at the 1st, 3rd and 5th hour during the incubation. The survival rate significantly decreased parallelly with the storage length in all the tested extenders. The total mean values sperm motility was 39.66 %, 30.77 %, 28.55 %, 28.67 % and 24.50 %, respectively, in A, AS, AS^+, M III and LD. The total mean value motility observed of Androhep was significantly ($P<0.001$) higher than of the others extenders. In conclusion, the results of this study showed that Androhep was a better extender than Androstar, M III, Androstar plus and LD in terms of survival rate of boar spermatozoa for long-term liquid preservation.

Key Words: Boar semen; long-term liquid extender, long-term thermo-resistance survival test; storage

Artificial insemination (AI) is an important tool in animal production (Vishwanath, 2003) and the sperm quality is one of the factors that determine successful insemination. More than 99 % of the estimated 19 million inseminations of gilts conducted world-wide use extended semen in a liquid state, which were stored at 15-20ºC for several days until used for AI (Huo et al., 2002). Semen used for AI is typically collected from boars and is ultimately diluted in any one of a variety of commercially available extenders. These extenders are used to create multiple insemination doses from a single ejaculate and contain buffers and nutrients that provide spermatozoa with an environment that maintains viability for three or more days of post-collection (Kuster and Althouse, 1999). Several studies have showed influence of different extenders on spermatozoa survival (Vyt et al., 2004) and boar sperm quality (Huo et al., 2002; Ambrogi et al., 2006; Waterhouse et al., 2004). It is known that short-term extenders (<3 days) are widely used but long-term extenders (>3 days) are interesting because they must preserve not only sperm cell viability but also sperm motility for the required period. Sperm motility is an indication of an active metabolism and the integrity of membranes (Johnson et al., 2000) and it is fundamental factor to the success of reproduction because it allows the sperm to reach the site of fertilization (Olds-Clarke, 1996) and penetrate the oocyte (Strauss et al., 1995).

The objective of this study was to compare preservation ability of long-term commercial extenders by means of spermatozoa long-term thermo-resistance survival test.

Material and Methods

Twenty-one ejaculates from 21 fertile hybrid boars of various ages (1 to 3 years) and coming from one AI centre in the Czech Republic were used in this study. All boars were kept under the same housing, feeding, and breeding conditions. Ejaculates were collected by a gloved-hand technique and the gel portion was removed using double gauze.

The following semen quality parameters were evaluated in samples of ejaculate: semen volume, sperm motility, percentage of viable spermatozoa, sperm concentration, morphologically abnormal spermatozoa and total number of spermatozoa per ejaculate. Sperm 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. Percentage of viable 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 and 2 drops of 10% nigrosin were added after 30 s. Two hundred spermatozoa per slide were evaluated under a light microscope (1500x). Sperm concentration was estimated using a Bűrker 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 diluted (dilution ratio 1+4) in a commercial boar semen extender Androhep (A), Androstar (AS), Androstar plus (AS^+), M III (Minitüb, Germany), LD (Magapor, Spain) and was stored at a temperature 17°C up to 96 h. The test was performed on 3 ml samples kept at 38°C in water bath each day and motility of spermatozoa was evaluated at the 1st, 3rd and 5th hour during the incubation.

Basic statistical characteristics of the results (arithmetic means, standard deviations, percentage and significance) were calculated using the QC Expert program (TriloByte Statistical Software, s.r.o., Pardubice, Czech Republic). 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 and Discussion

The initial quality of semen used in this study was as follows (mean ± SD): semen volume 281.33±119.39 ml; sperm motility 70.24±8.79 %; viable spermatozoa 68.05±9.20 %; sperm concentration 331.24±154.71x10^3/ mm^3; morphologically abnormal spermatozoa 26.55±19.81 % and total number of spermatozoa per ejaculate 87.18±46.51x10^9.

The effects long-term extenders were evaluated by means of spermatozoa long-term thermo-resistance survival test. Mean values sperm motility in single hours monitored - 1 h, 3 h and 5 h during long - term thermo-resistance survival test and after storage time 24h,48h, 72h and 96h from dilution of semen are presented in Table 1.

The mean motility of spermatozoa in native semen was 70 % and the mean motility after 1h monitoring was decreased by 20.24 % in A, 22.62 % in AS, 25.95 % in AS^+, 23.57 % in LD and 25 % in MIII after 24h storage time. The highest percentage of sperm motility was found in extender A and extender LD had the highest difference in low sperm motility in comparison to A. The decrease of sperm motility in extenders was observed from 3h to 5h than at 1h monitoring.

Comparison of mean values for motile spermatozoa in extenders and storage periods during long-term thermo-resistance survival test shows Table 2.

Sperm motility was decreased for all extenders with storage periods. A significant decrease in sperm motility was observed in A after 48h ($P < 0.05$) and in extenders AS, AS^+, MIII ($P < 0.001$) and LD ($P < 0.01$) after 72h of preservation. The effects of extenders on sperm motility are evident from total means where significant differences were recorded in the motility between extenders A vs. AS, AS^+, LD, MIII ($P < 0.001$) and AS vs. LD ($P < 0.05$). Ambrogi et al. (2006) also obtained similar results where low motility of sperm after 72h storage would be significantly influence of fertilizing ability of sperm. Kommisrud et al. (2002) found significant differences of motility after 78h and 102h. Extender A agreed with fifteen boars and extender LD was unsuitable for eleven boars according to the total sperm motility in long-term thermo-resistance survival test.

Figure 1 shows the effects of extenders and their influence on boar spermatozoa during long-term thermo-resistance survival test. The results found that extender A was not outdone by the other tested extenders ($P < 0.001$). Věžník et al. (2003) also recorded that extender A had the highest sperm motility from all tested extender.

Table 1. Mean values of sperm motility (%) in different extenders during long-term thermo-resistance survival test (n=21)

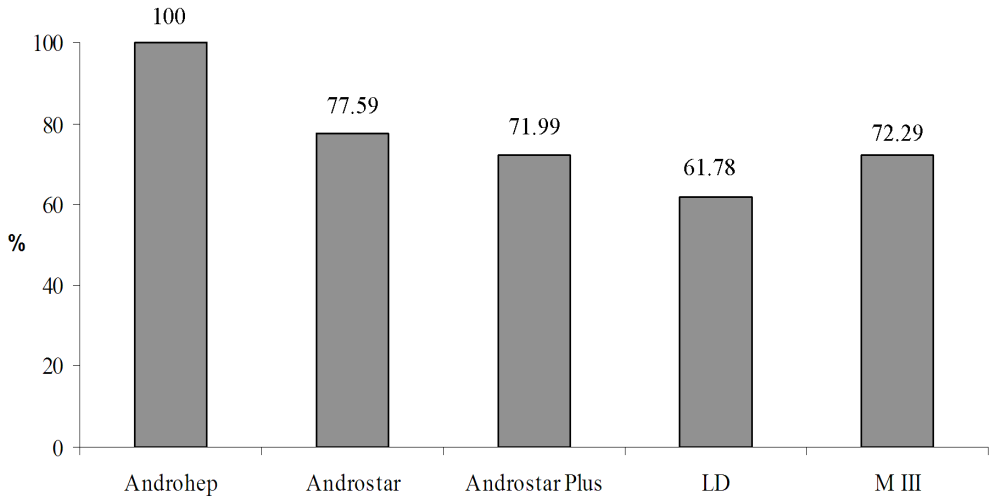
Extender	24h			48h			72h			96h		
	1h	3h	5h	1h	3h	5h	1h	3h	5h	1h	3h	5h
Androhep	50.00	47.86	43.10	50.24	43.33	38.10	46.67	36.90	30.71	39.52	28.57	20.95
Androstar	47.62	36.43	28.10	46.19	31.43	24.05	41.67	26.90	19.29	31.67	21.90	14.05
Androstar Plus	44.29	34.76	26.90	42.38	30.00	23.57	38.10	25.24	15.95	30.95	18.10	12.38
LD	46.67	30.24	16.19	41.90	24.05	13.81	37.86	17.62	12.14	28.57	15.24	9.76
M III	45.24	34.76	26.67	41.9	32.86	25.00	37.38	23.33	18.10	27.86	17.86	13.10

Table 2. Comparison of mean values for sperm motility (%) in different extenders during storage periods in long-term thermo-resistance survival test (n=21)

Extender	24h	48h	72h	96h	Total
Androhep	$46.98^{a,A}$	$43.89^{a,A}$	$38.10^{a,B}$	$29.68^{a,D,C}$	39.66^{a}
Androstar	37.38^{A}	33.89^{A}	29.29	$22.54^{D,B}$	$30.77^{d,a}$
Androstar Plus	$35.32^{b,A}$	$31.98^{b,A}$	26.43^{b}	$20.48^{D,B}$	28.55^{d}
LD	$31.03^{d,A}$	26.59^{d}	22.54^{d}	$17.86^{b,C}$	$24.50^{d,b}$
M III	$35.56^{b,A}$	33.25^{A}	26.27^{b}	$19.60^{D,C}$	28.67^{d}

^{a,b,c} means within the column ^{a,b} $P < 0.05$, ^{a,c} $P < 0.01$, ^{a,d} $P < 0.001$
^{A,B,C} means within the row ^{A,B} $P < 0.05$, ^{A,C} $P < 0.01$, ^{A,D} $P < 0.001$

Figure 1. Comparison of preservation effects of extenders to Androhep (100 %) in long-term thermo-resistance survival test



Conclusion

The results of this study showed that Androhep was a better extender than Androstar, M III, Androstar plus and LD in terms of survival rate of boar spermatozoa for long-term liquid preservation.

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