EFFECT OF THE NEW LIQUID CONCENTRATE EXTENDER ON THE BOAR SEMEN QUALITY COMPARED TO THE TRADITIONAL BTS EXTENDER

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Abstract

The objective of this study was to evaluate the boar semen quality in newly development liquid extender concentrate K2 compared to traditional BTS extender. Fifteen sperm rich fractions from 5 boars from one AI centre with motility ≥80 % and the number of morphologically abnormal spermatozoa ≤25 % were used in this study. Semen were diluted 1+4 in extenders K2 and BTS and stored at 17°C up to 72h storage time. The boar semen quality was evaluated by sperm motility, sperm viability, short hypoosmotic swelling test (sHOST) and long-term thermoresistance survival test (TRT). In total progressive motility were recorded differences between BTS (76.75 %) and K2 (67.65 %) extender ($P<0.05$). In sperm viability and sHOST were not found differences between extenders ($P>0.05$). In TRT test were noted differences ($P<0.05$) in sperm motility between BTS and K2 at 24h (55.24 % and 75.71 %) and 48h storage time (52.86 % and 63.33 %), in 1h (64.52 % and 75.48 %) and 5h (38.33 % and 50.24 %) of monitoring and in total sperm motility (52 % and 61 %). Boar semen quality were effected by extenders, storage time and hours of monitoring ($P<0.05$). In conclusion, according results is suitable used K2 extender into 48h storage time because than sperm motility is decreased. Further was found that BTS extender had worse thermoresistance stability in TRT test during monitoring hour compared to K2.

Key Words: Boar semen, extender, storage time, sperm quality

Artificial insemination (AI) is the most widely used reproductive technology in the porcine industry. Liquid preservation in short-term extenders is still the preferred method of boar semen storage. The function of extender is prolong sperm survival, to provide energy and nutrients needed for the metabolic maintenance of the sperm cells, to control pH, osmotic pressure of medium and to avoid the growth of bacteria (Bresciani et al., 2013). Preparation of extenders and the quality of insemination dose is one of the factors that participate in successful insemination. We tested the newly development liquid extender concentrate as a short-term extender which have simplify and shorted of time the preparation of insemination doses. Evaluation boar semen quality is importance for determining suitable extenders for preparing insemination doses. In this study was evaluated boar sperm quality by sperm motility, sperm viability and were applied short hypoosmotic swelling test (sHOST) which assesment the functionality of the sperm membrane and long-term thermoresistance survival test (TRT) which monitors sperm survival or the sperm´s ability to survive in the sow´s reproductive tract and retain their fertility (Fisher et al., 1991). Long-term TRT test is important indicator for assessing the biological value and sperm fertilization capacity. The objective this study was to evaluation of boar semen quality in newly development liquid extender concentrate K2 compared to traditional BTS extender.

Material and Methods

Fifteen sperm rich fraction with motility ≥80 % and number of morphologically abnormal spermatozoa ≤25 % from five fertile boars of Přeštice black-pied pigs aged 3.5 to 5 years were collected using the gloved-hand technique. The boars were kept in the same housing, feeding and breeding conditions.

The following parameters were evaluated in fresh native boar semen: semen volume, sperm motility, sperm concentration, morphologically abnormal spermatozoa, percentage of viable spermatozoa, sHOST, pH and osmolality. The sperm motility was assessed subjectively using phase contrast microscopy with a heating stage (38°C) at 200x magnification. Sperm concentration

was measured with Chroma Colorimeter 254 (Sherwood Scientific Ltd., Cambridge, England). Morphologically abnormal spermatozoa (MAS) were assessed according to the staining method of Čeřovský (1976) and evaluated microscopically under oil immersion and 1500x magnification. The percentage of viable spermatozoa was estimated by the supra-vital staining technique using the eosin-nigrosine stain mixture. One drop from each sample was mixed with 1 drop of 1% eosin Y and 2 drops of 10% nigrosine were added after 30s. Two hundred spermatozoa per slide were evaluated under a light microscope (1500x). Viable spermatozoa remain unstained white and dead cells are stained red. sHOST was assessed by the method according to Pérez-Llano et al. (2003) using the eosin-nigrosine staining technique. Sperms were incubated at 38°C for 5 min, with hypoosmotic solution (75mOsm/kg). At least 200 spermatozoa were evaluated per slide. The results of sHOST were included in four categories. sHOST positive (coiled tail) with negative head (white) was defined in this study. pH was assessment using Hanna precision pH meter at 20°C (Sigma-Aldrich, the Czech Republic) and osmolality (mOsmol/kg) with Marcel Osmometr OS 300 (2THETA ASE, Czech Republic).

The boar semen was diluted according to Čeřovský (1982) in dilution rate was 1 + 4 in BTS (Minitüb, Germany) and liquid extender concentrate K2 (VÚŽV v.v.i., Czech Republic). Samples were stored at a temperature of 17°C up to 72h. Sperm motility and kinetic indicators were estimated with the use Computer Assisted Semen Analysis (CASA). The sperm motility indicators were total sperm motility (%) and progressive motility (%). Kinetic indicators of speed were curvilinear velocity-VCL (µm/s), straight line velocity-VSL (µm/s), average path velocity-VAP (µm/s), linearity-LIN (VSL/VCL×100) and straightness-STR (VSL/VAP×100) and wobble-WOB (VAP/VCLx100). Sperm motility, percentage of viable spermatozoa, sHOST and long-term TRT test were evaluated at 24h, 48h and 72h storage time. Osmolality and pH were evaluated at 0h and 72h storage time. Long-term TRT test was performed on 3 ml samples kept at 38°C in water bath and motility was evaluated at the 1st, 3rd and 5th hour during incubation in storage time.

Basic statistical characteristics of the results arithmetic means, standard deviations (SD) and significance (P) were calculated by the QC Expert program (TriloBite Statistical Software s.r.o., Pardubice, Czech Republic). Statistical significance ($P<0.05$) was determined using of ANOVA-Fisher test.

Results and Discussion

The initial quality of semen was as follows native sperm motility 82.14 %, sperm concentration $382.14\times10^3/mm^3$, MAS 22.64 %, sperm viability 85.58 %, sHOST 63.67 %, pH 7.54 and osmolality 316.42 mOsmol/kg. Values of pH and osmolality extenders are presented in Table 1. There were differences between extender at 0h and 72h. The osmolality K2 was higher value which it could be due to the higher concentration of the main diluent components. Schilling and Vengust (1986) reported that values of motility and viability of spermatozoa will not only be influenced by the osmolality or extenders but also by other ingredients such as non-electrolytes, protecting colloids, energy compounds etc.;. Comparison of total results of sperm motility and kinetic indicators with the CASA system in BTS and K2 are recorded in Figure 1. There were recorded differences ($P<0.05$) only in total progressive motility of sperm between BTS and K2 (76.75 % and 67.65 %). In Figure 2 are noted results of sperm viability and sHOST between BTS and K2. There were not found differences between extenders ($P>0.05$). Karunakaran et al. (2017) mentioned that total motility and progressive motility was reduced significantly ($P<0.01$) in tested extenders during storage period. On the other hand Ambrogi et al. (2006) reported no effects of extenders by day on total motility, linear motility, VSL, VAP and VCL in short-term extender and differences between extenders in sperm viability, acrosome integrity and HOST positive did not found Sa et al. (2013) during 96h storage time. On the other hand Karunakaran et al. (2017) noted decreased functional membrane integrity between tested extenders and Khan et al. (2006) found significant difference in HOST test after 48h of preservation in observed extenders. Results of the long-term TRT test are shown in Figure 3. and in Figure 4. Statistical differences ($P<0.05$) in sperm motility in TRT test were noted between BTS and K2 at 24h (55.24 % and 75.71 %) and 48h (52.86 % and 63.33 %), in 1h (64.52 % and 75.48 %) and 5h (38.33 % and 50.24 %) of monitoring and in total sperm motility (K2 60.95 % vs. BTS 52.38 %). Extender K2 had the biggest decrease of sperm

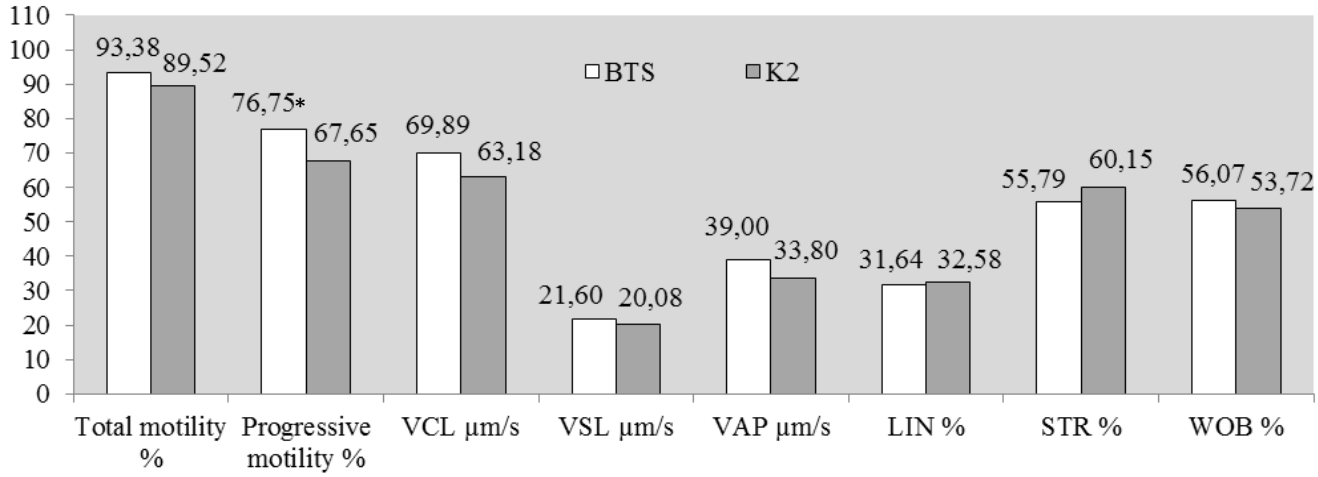
motility after 48h storage time and BTS after 3h long-term TRT test. Lange-Consiglio et al. (2013) used long-term TRT test in their study, where in all¨ samples were found motility reduction gradual over time (P<0.05) and it was evident between 4-8hours of incubation. Boar semen quality were effected by extenders, storage time and hours of monitoring in this study (P<0.05).

Table 1. Osmolality (mOsmol/kg) and pH in BTS and K2 at 0h and 72h storage time

Extender	Osmolality (mOsmol/kg)		pH	
	0h	72h	0h	72h
BTS	326.20±3.55^{A}	335.13±7.71^{A}	7.34±0.31^{a}	7.84±0.22^{b}
K2	473.40±18.49^{a,B}	493.20±24.35^{b,B}	7.22±0.19^{a}	7.75±0.35^{b}

^{A,B} means within column ^{A,B} P<0.05
^{a,b} means within the row ^{a,b} P<0.05

Figure 1. Comparison of total results of sperm motility and kinetic indicators with CASA systems in BTS and K2



*P<0.05

Figure 2. Comparison of total result of sperm viability and sHOST between BTS and K2

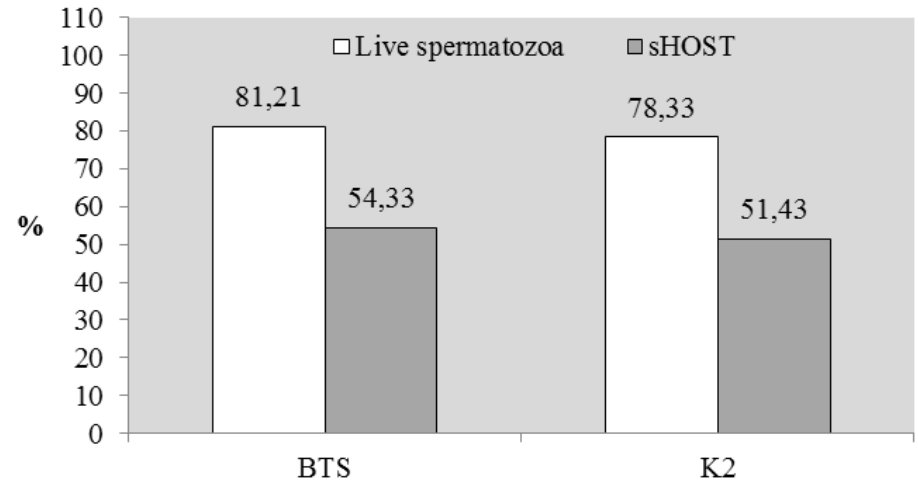
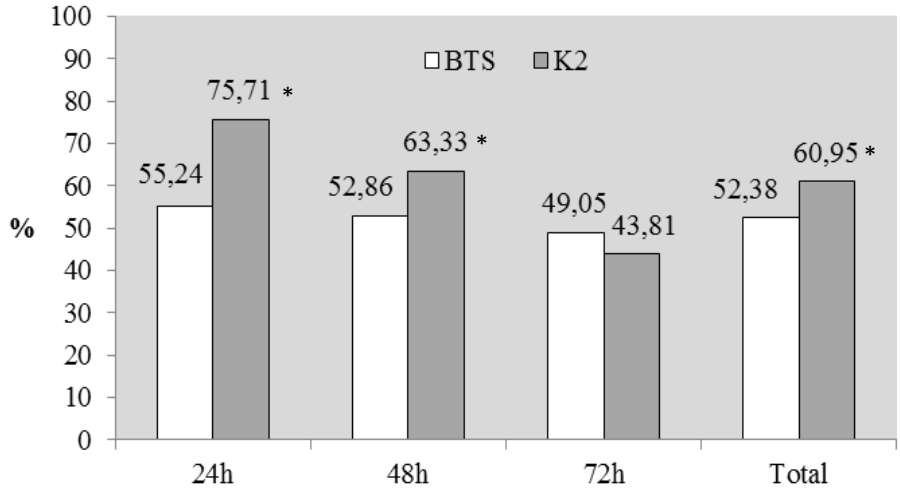
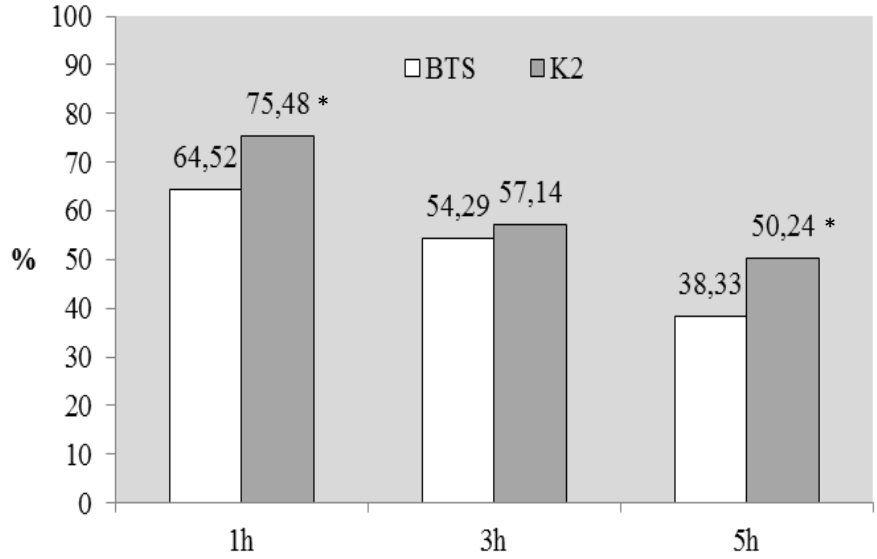


Figure 3. Comparison of total result of sperm motility in long-term TRT test between BTS and K2 during storage periods



*P<0.05

Figure 4. Comparison of total result of sperm motility in long-term TRT test between BTS and K2 during monitoring hours



*P<0.05

Conclusion

This study was compared the newly development extender K2 to the standard BTS extender with using various diagnostic laboratory techniques for assessment the boar semen quality. Results showed that K2 extender is suitable for used within 48h because than sperm motility is decreased. Further was found that BTS extender had worse thermo-resistance stability in TRT test during monitoring hour in comparison K2.

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