SPERM MOTILITY AND VIABILITY IN BOAR SPERM DILUTED IN LONG-TERM EXTENDER CONTAINING DIFFERENT TYPE OF KOFOLA

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Abstract

The objective of this study was to evaluate the sperm motility and viability of boar sperm diluted in long-term extender containing different types of kofola. Eight sperm rich fractions from 4 fertile boars from one AI centre with motility ≥80% and the number of morphologically abnormal spermatozoa ≤25% were used in this study. Tested types of kofola: mix of spices – KS (cinnamon extract 100mg/l and clove extract 100mg/l) and honey – KH (honey extract 100mg/l) were added to boar semen extender Androstar plus (AS+). Semen was diluted in AS+ as a control (K) and AS+ with addition of different types of kofola in the dilution ratio 1+2. Samples were stored at 17°C and evaluated after 0h, 48h, 92h and 168h storage time for sperm motility and sperm viability. SCA system was used for determined sperm motility (total, progressive, non-progressive and immotile) and kinetic characteristics - straight line velocity (VSL, μm/s), average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s), straightness (STR, %), linearity (LIN, %), wobble (WOB, %). Statistically significant differences in total mean values of total sperm motility, progressive and immotile sperm ($P<0.05$) were recorded between K (94.47, 73.91, 5.53%) vs. KH (85.58, 59.85, 14.43%) and KS (87.10, 60.96, 12.91%). The sperm survival was significantly affected by storage time ($P<0.05$). Kinetic indicator of speed VCL and LIN reported significant differences between K (57.73 μm/s, 36.16%) vs. KH (49.10 μm/s, 39.52%) $P<0.05$. No differences were found in percentage of sperm viability ($P>0.05$) between K (77.75%) vs. KH (77.88%) and KS (79.81%). In conclusion, application of tested types of kofola as a potential component to the boar extender unfortunately did not have a positive effect on boar sperm motility, but the sperm viability was not adversely affected.

Key Words: Boar semen, extender, kofola, honey extract, cinnamon extract, clove extract, sperm motility, sperm viability

Artifical insemination is an important tool in animal production (Vishwanath, 2003). The effective use of semen for AI depends upon the ability of extender to provide a suitable environment for spermatozoa during storage. Extenders can be classified as short term (3 days), midterm (4-5 days) and long term (7 days) (Gadea, 2003). Two main factors influence sperm cell function after ejaculation and during in vitro storage: the temperature at which the semen is collected and stored after dilution and conditions of the suspension medium (Johnson et al., 2000). The goal of extenders is to prolong sperm survival, supply sperm energy, balance pH and prevent bacterial growth (Vyt et al., 2004). Akandi et al. (2015) demonstrates that spermatozoa can be stored in extenders containing honey, sugarcane juice, tomato juice and pineapple juice.

The objective of this study was to evaluate the survivability of boar sperm diluted in long-term extender containing different types of kofola.

Material and Methods

Eight sperm rich ejaculate fraction with motility ≥80% and number of morphologically abnormal spermatozoa ≤25% from four fertile AI boars of Přeštice black-pied pig aged 3.5 to 5 years were collected using the gloved-hand technique. The boars came from the boars 'insemination station in the Czech Republic where were kept in the same housing, feeding and breeding conditions.

The following parameters were evaluated in the fresh native boar semen: semen volume, sperm motility, sperm concentration, morphologically abnormal spermatozoa sperm, viability and short hypoosmotic swelling test (sHOST). The sperm motility was assessed subjectively using phase contrast microscopy with a heating stage (38°C) at 200× 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 1500× magnification. 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, then 2 drops of 10% nigrosine were added after 30s. Two hundred spermatozoa per slide were evaluated under a light microscope (1500×). 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.

The boar semen was diluted in dilution rate 1+2 in extender Androstar plus 93.75% (AS+, Minitüb, Germany) with kofola 6.25% stored at 17°C and evaluated at 0h, 48h, 92h and 168h and compared with control sample with AS^+ in dilution rate 1+2 (K). Tested types of kofola (Kofola a.s., Krnov, Czech Republic): kofola mix of spices – KS (cinnamon extract 100mg/l and clove extract 100mg/l) and kofola honey – KH (honey extract 100mg/l). This selected amount of different types of kofola had no effect on initial sperm motility ($P<0.05$) unlike higher amount different types of kofola.

Sperm motility (total, progressive, non-progressive and immotile) and kinetic characteristics – straight line velocity (VSL, μm/s), average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s), straightness (STR, VSL/VAP, %), linearity (LIN, VSL/VCL, %), wobble (WOB, VAP/VCL, %) were assessed using SCA software (Sperm Class Analyzer, version 5.4. Microptic S.L., Spain). Evaluation was performed using a 2 μl sample placed in a Leja 20 chamber and 500 sperm were evaluated by negative phase contrast microscopy with a heating stage (38°C) at 160× magnification.

Basic statistical characteristics of the results of 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 was checked by the analysis of variance ANOVA - Fisher test at significance level of $P<0.05$.

Results and Discussion

The initial quality of native semen was as follows (mean±SD): semen volume 222.50±69.63ml, sperm motility 85.00±0.00%, sperm concentration $497.83±44.85×10^3/mm^3$, MAS 18.58±6.21%, sperm viability 73.42±7.34% and sHOST test 53.60±12.14%.

The results of boar sperm motility are shown in Figure 1. There are total mean values of total sperm motility, progressive sperm motility, non-progressive sperm motility and immotile sperm. Statistically significant differences ($P<0.05$) were recorded between K vs. KS and KH in total sperm motility (94.47 vs. 87.10, 85.58%),

progressive sperm motility (73.91 vs. 60.96, 59.85%) and in immotile sperm (5.53 vs. 12.91, 14.43%). Broekhuijse et al. (2012) concluded that sperm motility is considered to be an important parameter with which to validate the quality of an ejaculate.

More detailed determinations of progressive motility in the analyzed samples K, KS and KH during the determination of 0h, 48h, 96h and after 168h are recorded in Figure 2. Kommisrud et al. (2002) found in their study significant differences in sperm motility after 78h and 102h storage time. Motility of < 60% negatively affected all fertility parameters (sperm penetration rate in vitro, farrowing rate and litter size (Jung et al., 2015).

In progressive motility, statistically significant differences were found not only between different samples, but also for the same samples during the storage period ($P<0.05$). The sperm survival was significantly affected by storage time ($P<0.05$). Bielas et al. (2017) also noted that sperm characteristics, especially all motility and structural parameters were significantly affected by the storage time.

Total mean values of sperm movement are illustrated in the Figure 3. Kinetic indicator of speed VCL and LIN reported significant differences between K (57.73 μm/s, 36.16%) vs. KH (49.10 μm/s, 39.52%) $P<0.05$.

In the Figure 4 were not found differences ($P>0.05$) in percentage of sperm viability between K (77.75%) vs. KH (77.88%) and KS (79.81%). Similar results were also found Ambrogi et al. (2006) and Dubé et al. (2004) that sperm viability was not significantly affected by used extenders. Bielas et al. (2017) also noted that sperm viability was almost unchanged in long-term extender until 168h storage time.

Akandi et al. (2015) findings, survivability of boar sperm stored under room temperature can be maintained longer in honey and sugarcane juice extenders compared with tomato and pineapple extenders. We found in this study that sperm motility was lower compared to control about 13-14% and better results were obtained in KS compared to KH. There were no differences in sperm viability between samples.

Figure 1. Comparison of total mean values of sperm motility, progressive sperm motility, non-progressive motility and immotile sperm (%) between K, KS and KH

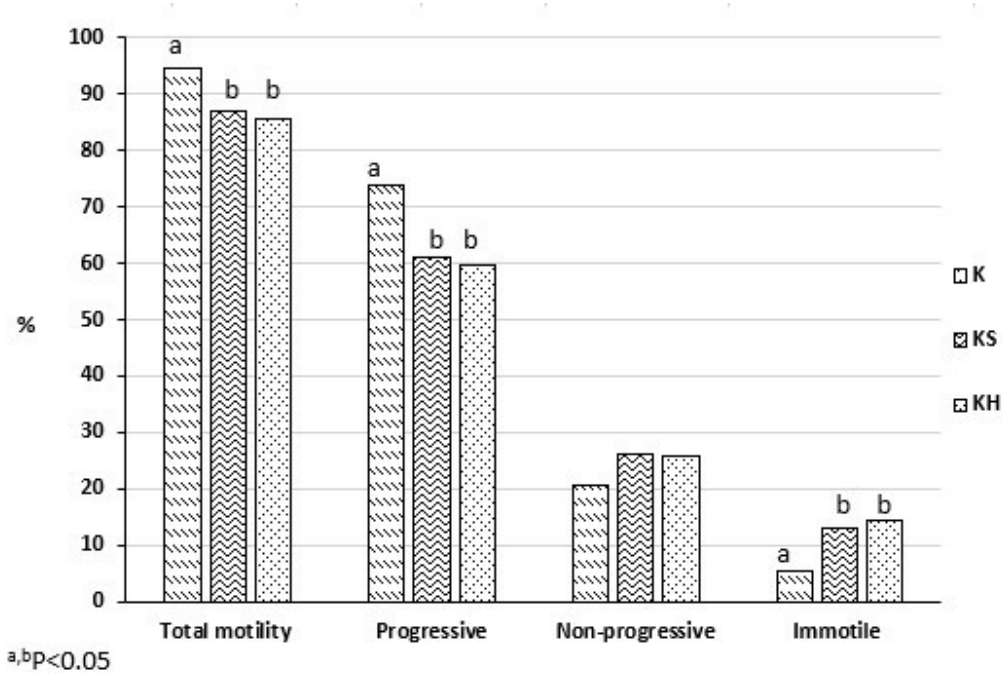
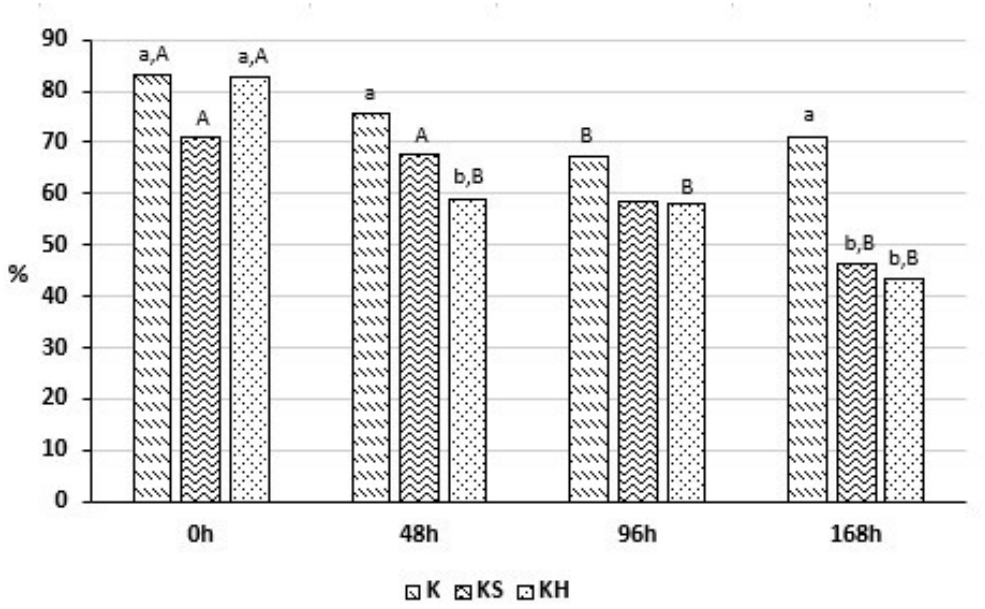
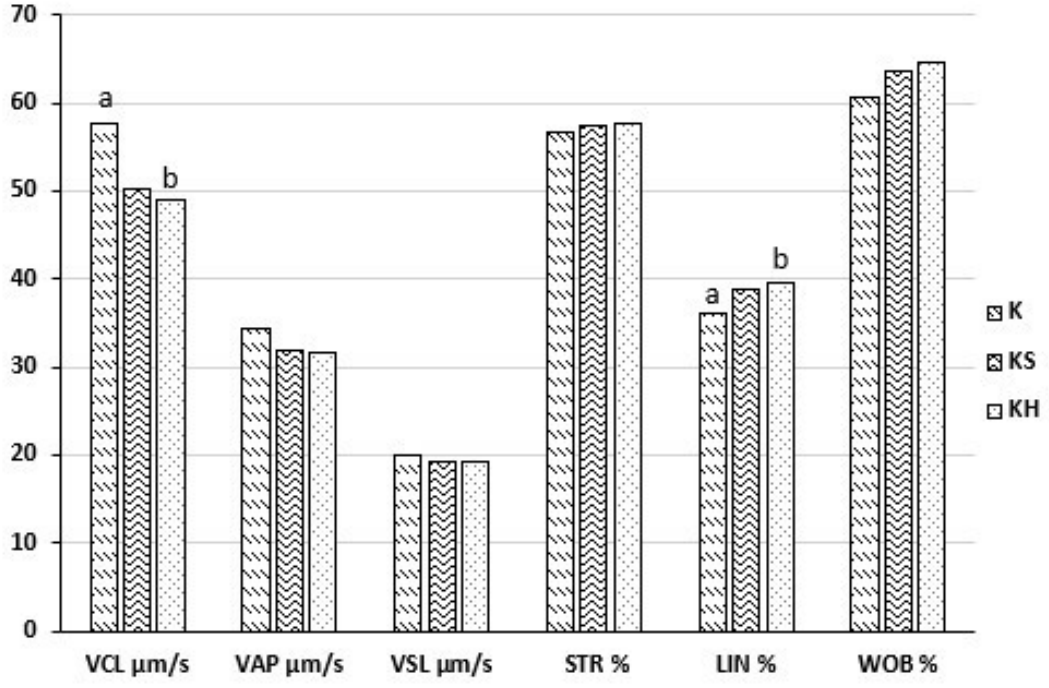


Figure 2. Comparison of mean values of progressive sperm motility (%) between K, KS and KH during evaluation at 0h, 48h, 96h and 168h



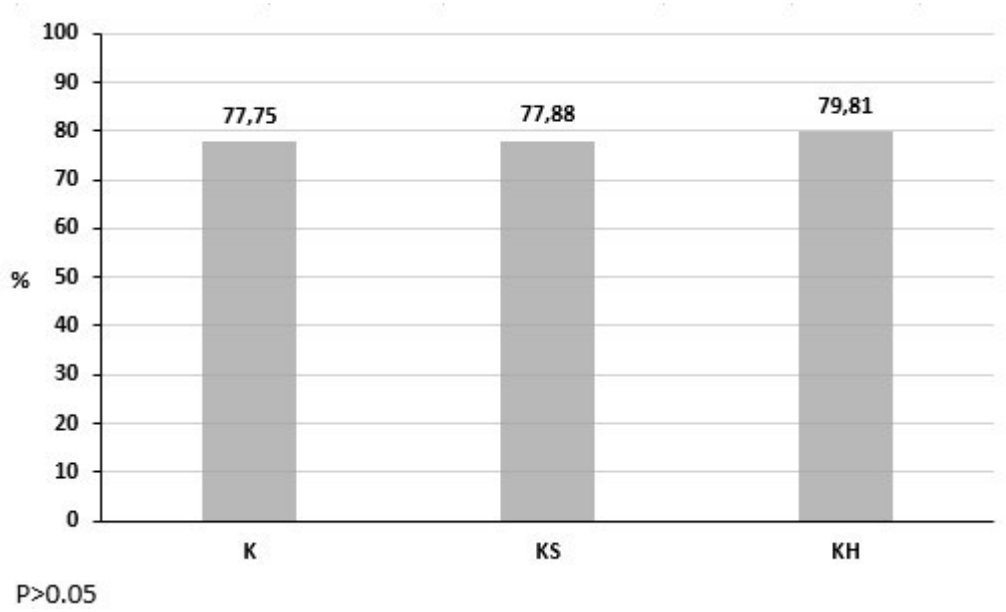
[a,b] different superscripts in the same hour of progressive motility evaluation indicated significant differences between K, KS and KH at P<0.05
[A,B] different superscripts between hours of progressive motility evaluation indicated significant differences between in the same sample K, KS and KH at P<0.05

Figure 3. Comparison of total mean values of sperm movement-kinetic characteristics between K, KH and KS



[a,b]P<0.05

Figure 4. Comparison of total mean values of sperm viability (%) between K, KS and KH



Conclusion

Application of tested types of kofola as a potential component to the extender unfortunately did not have a positive effect on boar sperm motility, but the sperm viability was not adversely affected. Therefore, it would be beneficial to look for other potential components alternative to improve the extender quality for boar sperm preservation.

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