SURVIVABILITY OF BOAR SPERM DILUTED IN LONG-TERM EXTENDER CONTAINING KOFOLA

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Abstract

The objective of this study was to evaluate the survivability of boar sperm diluted in long-term extender containing 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. Kofola original – KO was added to boar semen extender Androstar plus (AS+). Semen was diluted in AS+ as a control (K) and AS+ with KO 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, VSL/VAP, %), linearity (LIN, VSL/VCL, %), wobble (WOB, VAP/VCL, %). Statistically significant differences ($P<0.05$) were recorded between K (94.47, 73.91, 5.53%) vs. KO (87.76, 61.10, 12.24%) in total mean values of total sperm motility, progressive and immotile sperm. The progressive sperm motility was 13% lower in KO than K at 96h storage time then showed a sharp decrease after 168 h. The sperm survival was significantly affected by storage time ($P<0.05$). Between kinetic indicator of speed not reported significant differences between K vs. KO ($P>0.05$). No difference between K (77.75%) vs. KO (79.44) were found in percentage of sperm viability ($P>0.05$). In conclusion, the results of motility and viability point to the possibility of using kofola original as a potential ingredient in long-term boar extender until the storage time of 96 hours.

Key words: Boar semen, extender, kofola, sperm motility, sperm viability

Liquid preservation is still the preferred method of storing boar semen for AI in pig breeding. The effective use of semen for AI depends upon the ability of extender to provide a suitable environment for spermatozoa during storage. The function of extender is prolonging 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). It is known that short-term extenders (within 3 day) are widely used but long-term extenders (over 3 days) are interesting because they must preserve not only sperm cell viability but also sperm motility for the required period (Johnson et al., 2000). Akandi et al. (2015) demonstrates that spermatozoa can be stored in extenders containing honey, sugarcane juice, tomato juice and pineapple juice. So why not try adding kofola to boar semen extender.

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 t echnique.

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. (2001) 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 original KO (Kofola a.s., Krnov, Czech Republic) 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). This selected amount of KO had no effect on initial sperm motility ($P<0.05$) unlike higher amount of KO.

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. KO in total sperm motility (94.47 vs. 87.76%), progressive sperm motility (73.91 vs. 61.10%) and in immotile sperm (5.53 vs. 12.24%). Sperm motility is an indication of the active metabolism and integrity membrane and stored semen should by examined daily, with motility values above 60% considered satisfactory (Johnson et al., 2000).More detailed determinations of progressive motility in the analyzed samples K and KO during the determination of 0h, 48h, 96h and after 168h are recorded in Figure 2.

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 best values of progressive motility were found in the sample K vs. KO up to 96h storage time. KO is possible to used only until 96 storage time, when the progressive sperm motility was 13% lower than in K. The KO sample then showed a sharp decrease in progressive motility after 168 hours of storage time. Kommisrud et al. (2002) found in this study significant differences in sperm motility after 78h and 102h storage time.

Total mean values of sperm movement are illustrated in the Figure 3. Between kinetic indicator of speed not reported significant differences between K vs. KO (P>0.05).

In the Figure 4 were not found differences (P>0.05) in percentage of sperm viability between K (77.75%) vs. KO (79.44%). Similar results were also found Ambrogi et al. (2006) and Dubé et al. (2004) that sperm viability was not significantly affected by used extenders. We found that sperm viability was 1.69% higher in KO than in K.

We recorded in our study that is possible used kofola to boar semen extender up to 96h 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.

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

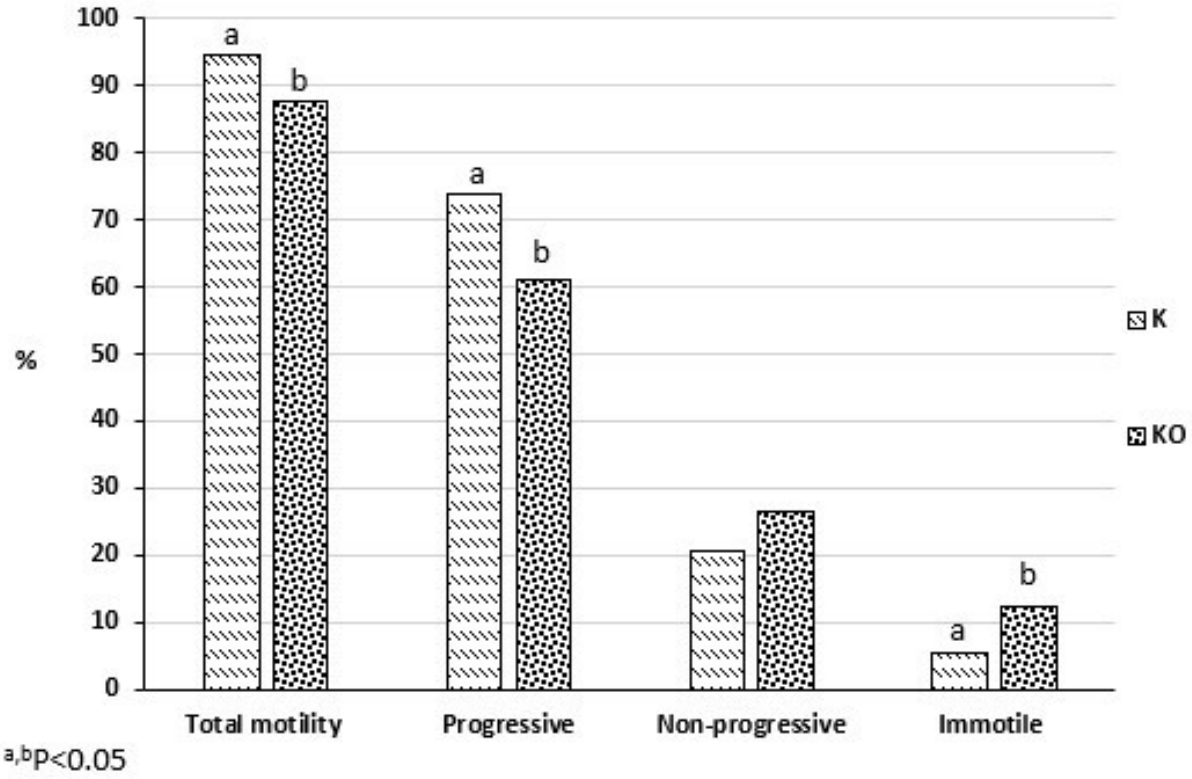
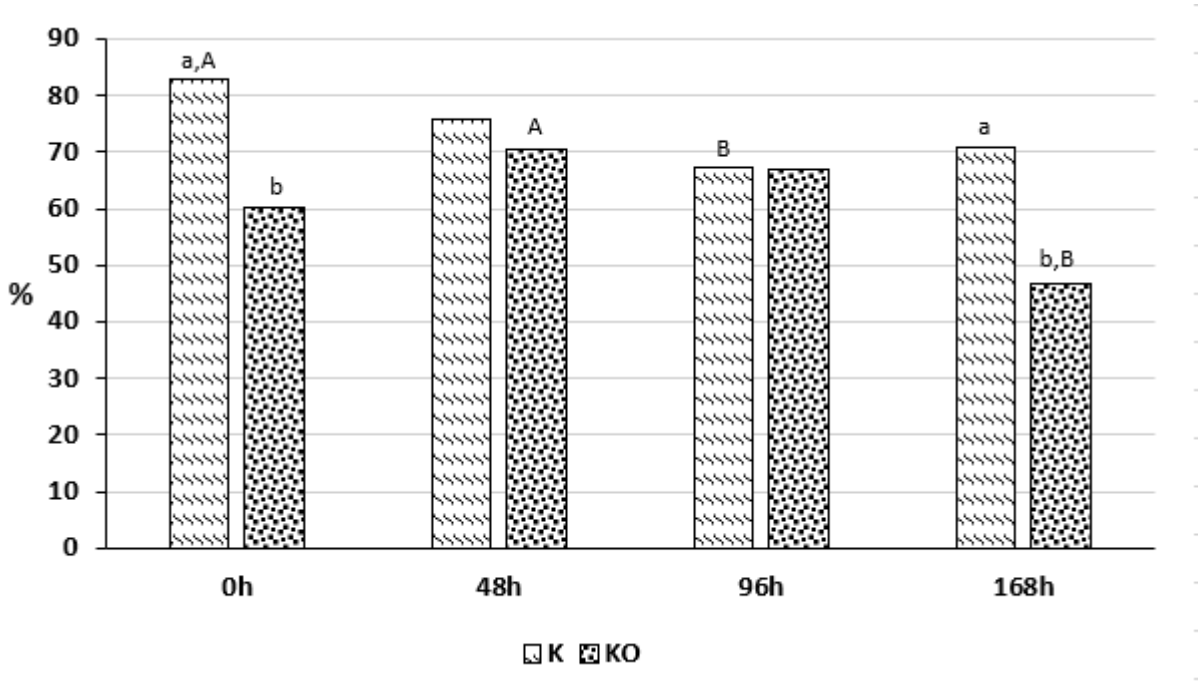
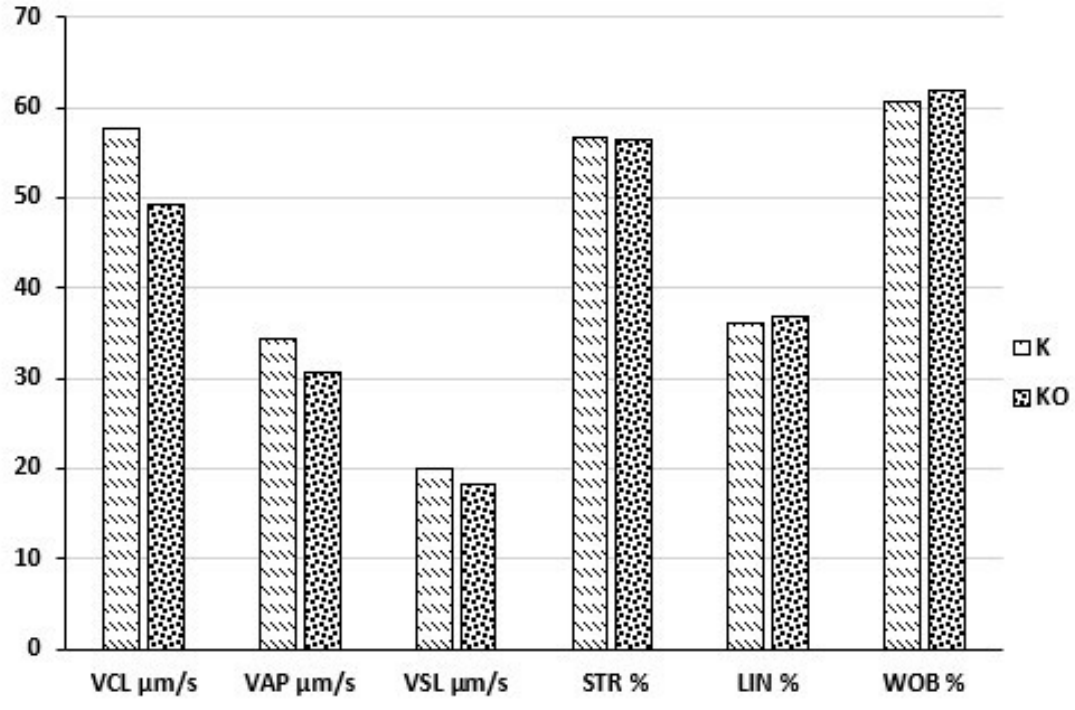


Figure 2. Comparison of mean values of progressive sperm motility (%) between K and KO during evaluation at 0h, 48h, 96h and 168h



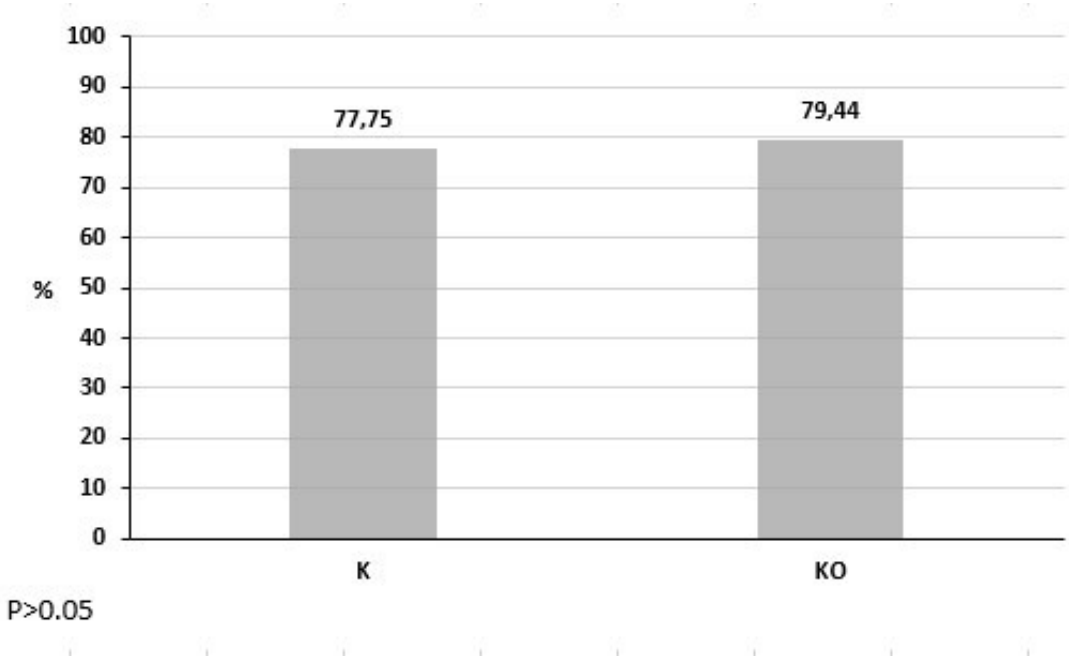
[a,b] different superscripts in the same hour of progressive motility evaluation indicated significant differences between K and KO at P<0.05
[A,B] different superscripts between hours of progressive motility evaluation indicated significant differences between in the same sample K and KO at P<0.05

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



[a,b]P>0.05

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



Conclusion

In conclusion, the results of motility and viability point to the possibility of using kofola original as a potential ingredient in long-term boar extender until the storage time of 96 hours.

References

AMBROGI DE M., BALLESTER J., SARAVIA F., CABALLERO I., JOHANNISSON A., WALLGREN M., ANDERSSON M., HERIBERTO RODRIGUEZ-MARTINEZ H. 2006. Effect of storage in short – and long -term commercial semen extenders on the motility, plasma membrane and chromatin integrity of boar spermatozoa. International Journal of Andrology, vol. 29, pp. 543-52, https://doi.org/10.1111/j.1365-2605.2006.00694.x.

AKANDI A., UGWU S. O., MACHEBE N. S. 2015. Survivability of boar sperm stored under room temperature in extenders containing some natural products. Open Access Animal Physiology, vol. 7, pp. 57-64, https://doi.org/10.2147/OAAP.S71360.

BRESCIANI C., MORINI G., BETTINI R., BIGLIARDI E., IANNI F. D., CABASSI C. S., SABBIONI A., PARMIGIANI E. 2013. Reproductive efficiency of a new modified boar semen extender for liquid storage. Livestock Science, vol. 257, pp. 384-388, https://doi.org/10.1016/j.livsci.2013.07.005.

ČEŘOVSKÝ J. 1976. Metoda barvení kančích spermií pro morfologické hodnocení. Živočišná Výroba, vol. 21, pp. 361-366.

DUBÉ CH., BEAULIEU M., REYES-MORENO C., GUILLEMETTE CH., BAILEY J. L. 2004. Boar sperm storage capacity of BTS and Androhep Plus: viability, motility, capacitation, and tyrosine phosphorylation. Theriogenology, vol. 62, pp. 874-86, doi: 10.1016/j.theriogenology.2003.12.006.

JOHNSON L. A., WEITZE K. F., FISER P., MAXWELL W. M. C. 2000. Storage of boar semen. Animal Reproduction Science, vol. 62, pp. 143-172, https://doi.org/10.1016/S0378-4320(00)00157-3.

KOMMISRUD E., PAULENZ H., SEHESTED E., GREVLE I. S. 2002. Influence of Boar and Semen Parameters on Motility and Acrosome Integrity in Liquid Boar Semen Stored for Five Days. Acta veterinaria Scandinavica, vol. 43, pp. 49-55, DOI:10.1186/1751-0147-43-49.

PÉREZ-LLANO B., LORENZO J. L., YENES P., TREJO A., GARCÍA-CASADO P. 2001. A short hypoosmotic swelling test for the prediction of boar sperm fertility. Theriogenology, vol. 56, pp. 387-398, https://doi:10.1016/S0093-691X(01)00571-4.

VĚŽNÍK Z., ŠVECOVÁ D., ZAJÍCOVÁ A. 2003. Repetitorium spermatologie a andrologie a metodiky spermatoanalýzy. Výzkumný ústav veterinárního lékařství, Brno, pp.161.

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