THE EFFECT OF VARIOUS SUBSTANCES ON INHIBITION OF MICROORGANISMS AND SPERM SURVIVAL IN SHORT-TERM BOAR SEMEN EXTENDER

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Abstract

The objective of this study was to investigate the effect of sodium thiosulfate (TS1, 2, 3), colloidal zinc + ascorbic acid (CZ1, 2) and honey (H) added to boar semen extender BTS without antibiotic (BTS0) as a substitute for antibiotics on inhibition of microorganisms and their influence on sperm survival. The amount tested substances added to boar semen extender was not effect on initial sperm motility ($p<0.05$) unlike higher amount of substances. Nine ejaculates from 4 healthy and fertile AI boars were used for this study. Tested substances were added to BTS0 extender in dilution rate of 1+2, 1+4 and 1+8 and stored at 17°C up to 48h, for everyday evaluation. Sperm survival was affected by storage time, dilution ratio and type of substances ($p<0.05$). Significant differences ($p<0.05$) of total mean values of sperm motility were found between samples BTS0 70.21% vs. BTS0+TS3 61.39% and BTS0+H 49.09%. The sample with the BTS0+H had statistically lower sperm motility 49.09% compared to other tested samples ($p<0.05$). Microorganisms were not significantly inhibited by any of tested substances added to the extender. In conclusion, utilization of tested substances as a potential substitute for antibiotics in boar semen extender is not possible. Therefore, it is necessary to research other potential substances for a possible replacement for antibiotic in boar semen extender.

Key Words: Boar semen, short extender, substances, microorganisms, sperm motility

Artificial insemination is a common preferred method of reproductive technology used in the pig breeding. Freshly ejaculated boar semen is usually contaminated by a wide range of microorganisms, which endangering sperm quality and fertility sperm (Maes et al., 2008; Maroto et al., 2010). Bacterial contamination of the boar semen is associated with a decrease in sperm motility, viability (Bussalleu et al., 2011) and membrane integrity (Sepúlveda et al., 2014). It was found that many microorganisms have become resistant to the most antibiotic used in semen extenders (Schulze et al., 2015; Morrell and Wallgren, 2014) and now are looking possible alternatives which could be used to reduce this resistance.

Zinc is one of the important trace elements in the body. Deficiency zinc is causes infertility and supplementation leads to improved fertility of animals, increasing concentration and sperm motility (Wroblewski et al., 2003). Zinc ions (Zn^{2+}) exhibit antimicrobial activity against various bacterial and fungal strains. The partial dissolution of zinc oxide (ZnO) particles releases Zn^{2+} ions in aqueous suspension that contributes to the antimicrobial activity of ZnO (Pasquet et al., 2014).

Honey is one of the oldest traditional medicines considered as traditional remedy for microbial infections (Brudzynski, 2006). Honey possesses therapeutic potential, including would the healing properties and antimicrobial activity (Mahendran et al., 2015). The antibacterial activity of honey is associated with strong osmotic effect and pH (Kwakman and Zaat, 2012). The main key role of the antimicrobial activity of honey is ability to produced hydrogen peroxide (Kacaniova et al., 2011).

The objective of this study was to investigate the effect of sodium thiosulfate, colloidal zinc + ascorbic acid and honey added to boar semen extender on inhibition of microorganisms and their influence on sperm motility.

Material and Methods

Nine 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 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, pH and osmolality. 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. The pH was assessment using the Hanna precision pH meter at 20°C (Sigma-Aldrich, Czech Republic) and osmolality (mOsmol/kg) with the Marcel Osmometr OS 300 (2THETA ASE, Czech Republic).

The boar semen was diluted in dilution rate 1+2, 1+4 and 1+8 in extender BTS and BTS without antibiotics - control samples (Minitüb, Germany). Sodium thiosulfate TS1 - 0.75g/l, TS2 - 1g/l, TS3 - 1.25g/l (Lachema Brno, Czech Republic), colloidal zinc + ascorbic acid CZ1 - 0.008 + 0.1g/l, CZ2 - 0.017 + 0.2g/l (Pharma Activ Czech s.r.o., Czech Republic) and honey (H) were added to in extender BTS without antibiotics (BTS0) in the dilution rate 1+2, 1+4, 1+8. This selected amount of substances added to boar semen extender was not effect on initial sperm motility ($p<0.05$) unlike higher amount of substances. Samples were diluted in the same dilution rate as a control samples and were stored at a temperature of 17°C up to 48h. Sperm motility was evaluated at 0h, 24h and 48h storage time. Sperm motility (%) was estimated with the use Computer Assisted Semen Analysis (CASA). For this study, value of sperm motility was expressed as a progressive sperm motility according CASA program. The pH was evaluated in all tested samples at 0h.

The assessment antibacterial activity of TS1, 2, 3, CZ1, 2 and H was in a microbiological laboratory. Each sample was diluted 100× in physiological saline solution (Penta s.r.o., Czech Republic) and then 100µl of the sample was inoculated on blood agar with 5% defibrinated ram blood (HiMedia Laboratories, USA). Samples were incubated for 48h at 37°C in a biological thermostat BT 120MR (EKOM s.r.o., Czech Republic). The number of colonies was determined by colony counter STC 1000 (VWR, Switzerland) and the total number of microorganisms was determined according to the formula and expressed in colony-forming unit (CFU/ml).

Basic statistical characteristics of the results of arithmetic means, standard deviations 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 ANOVA-Fisher test.

Results and Discussion

The initial quality of native semen was as follows: semen volume 259.56±82.59ml, sperm motility 83.33±2.5%, sperm concentration 379.33±80.69×10^3/mm^3, MAS 22.36±1.42 %, pH 7.87±0.26 and osmolality 315.29±12.26 mOsmol/kg. Values of pH and osmolality extenders are presented in the Table 1 where BTS0+H had lower values osmolality which could affect sperm motility. Table 2 shows pH of boar semen dilution samples with different amount of tested substances. Tested samples had the optimal pH for maintaining good motility and fertility during 48h storage time. Johnson et al. (2000) mentioned that in freshly ejaculated boar semen the pH varies between 7.2 and 7.5 and below this the motility and metabolism of spermatozoa are reduced gradually. Comparison of mean values of sperm motility (%) in samples with different tested substances and dilution rate to control sample BTS0 are presented in the Table 3. Sperm motility was affected by storage time, the dilution ratio and substances ($p<0.05$). Significant differences of total mean values of sperm motility were found between samples BTS0 70.21% vs. BTS0+H 49.09% and BTS0+TS3 61.39% ($p<0.05$). Honey had negative effect on sperm motility during storage time in all dilution rate compared to others. Comparison of total sperm motility in different tested substances to BTS0 as 100% is recorded in

Figure 1. Differences in sperm motility were between BTS0 and BTS0 + TS3 about 13 % and BTS0+H about 30 % ($p<0.05$).

The most common microorganisms found in boar semen samples: *E.coli, Proteus* sp., *Staphylococcus aureus, Staphylococcus cohnii* subsp. *Cohnii, Staphylococcus simulans, Staphylococcus cohnii* subsp. *Urealyticum, Corynebacterium* sp., *Bacillus* sp., *Moraxella canis, Chryseobacterium gleum.* A similar representation of microorganisms was reported by Bresciani et al. (2014) and Gaczarzewicz et al. (2016). The mean value of microorganisms in native boar semen was 2.2×10^4 CFU/ml at monitoring effect of TS on microorganisms (Figure 2). TS1, 2 and 3 did not inhibit microorganisms in samples.

Figure 3 shows the effect CZ. The mean value of microorganisms in native boar semen was 2.9×10^4 CFU/ml after the 3rd day was amount of microorganism uncountable (increase of E. coli and Proteus sp.). CZ1 and CZ2 also had not effect on decreased microorganisms during 48h.

The effect of H on number of microorganisms is presented in Figure 4 where the mean value of microorganisms in native boar semen was 2.5×10^4 CFU/ml. The effect of H also was not proved. Typical bacterial concentrations in the boar semen are presented range from 10^3 to 10^5 CFU/ml (Morrell and Wallgren, 2011). Microorganisms were not significantly inhibited by any of tested substances TS1, 2 and 3, CZ1 and 2 and H ($p>0.05$).

Conclusion

In conclusion, utilization of our tested substances as a potential substitute for antibiotics in boar semen extender is not possible because their low activity in the reduction of microorganisms was found. Therefore, it is necessary to research other potential substances for a possible replacement for antibiotics in boar semen extender.

Table 1. pH and osmotic activity of extenders

Extender	pH	osmotic activity (mOsmol/l)
BTS	7.94	324
BTS0	8.17	333
BTS0+TS1	7.82	326
BTS0+TS2	7.81	323
BTS0+TS3	7.83	329
BTS0+CZ1	7.87	309
BTS0+CZ2	7.80	312
BTS0+H	7.80	253

Table 2. pH of tested samples in different extenders and dilution rates

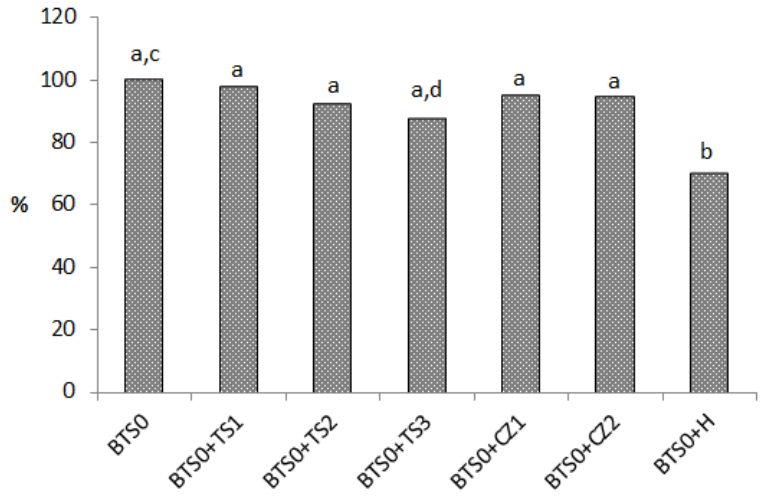
Extender	1+2	1+4	1+8
BTS	7.46	7.59	7.73
BTS0	7.31	7.56	7.66
BTS0+TS1	7.51	7.77	7.68
BTS0+TS2	7.43	7.67	7.65
BTS0+TS3	7.38	7.45	7.68
BTS0+CZ1	7.23	7.66	7.53
BTS0+CZ2	7.26	7.59	7.53
BTS0+H	7.41	7.65	7.84

Table 3. Comparison of mean values of sperm motility (%) in extender BTS0 with added different substances to control sample BTS0

Extender	1+2	1+4	1+8	Total
BTS	71.11	71.67[a]	68.33[a,c]	70.21[a,c]
BTS0	71.25	70.63	68.75[a]	70.37[a]
BTS0+TS1	70.01	68.33	67.50[a]	68.61[a]
BTS0+TS2	66.67	66.11	61.67[a]	64.81[a]
BTS0+TS3	63.33	63.33	57.50[a,d]	61.39[a,d]
BTS0+CZ1	68.33	68.33	63.33[a]	66.67[a]
BTS0+CZ2	68.33	66.11	64.17[a]	66.20[a]
BTS0+H	62.33[A]	51.40[b,B,C]	33.55[b,B,D]	49.09[b]

[a,b] means within row [a,b] p<0.05
[A,B] means within colum [A,B] p<0.05

Figure 1. Comparison of total sperm motility (%) of BTS0+TS1, 2, 3, BTS0+CZ1, 2 and BTS0+H to BTS0 (100%)



[a,b,c,d] p<0.05

Figure 2. Determination of the colony-forming unit (CFU/ml) of microorganisms in extender BTS0 with added TS1, 2, 3 and control samples (BTS, BTS0)

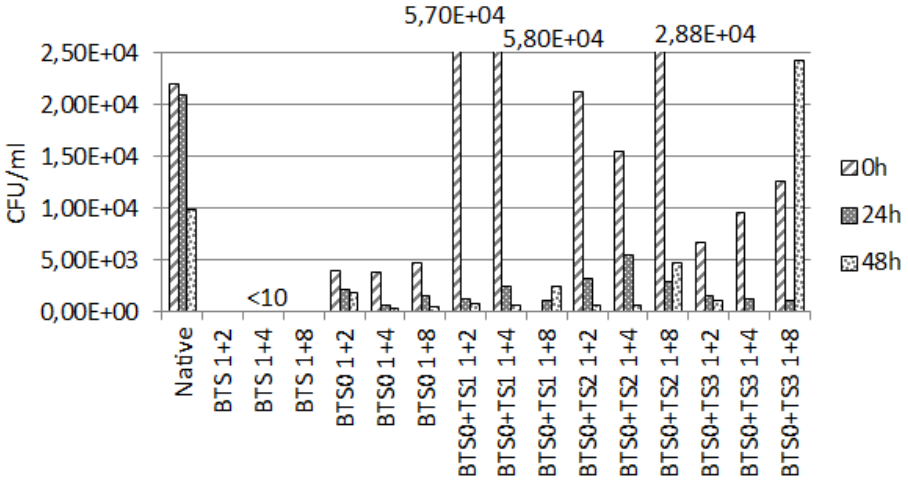


Figure 3. Determination of the colony-forming unit (CFU/ml) of microorganisms in extender BTS0 with added CZ1, 2 and control samples (BTS, BTS0)

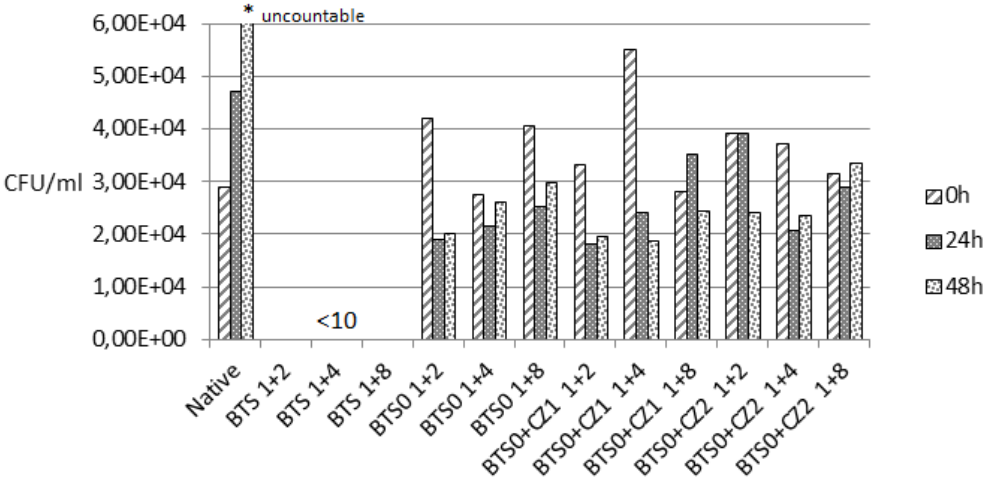
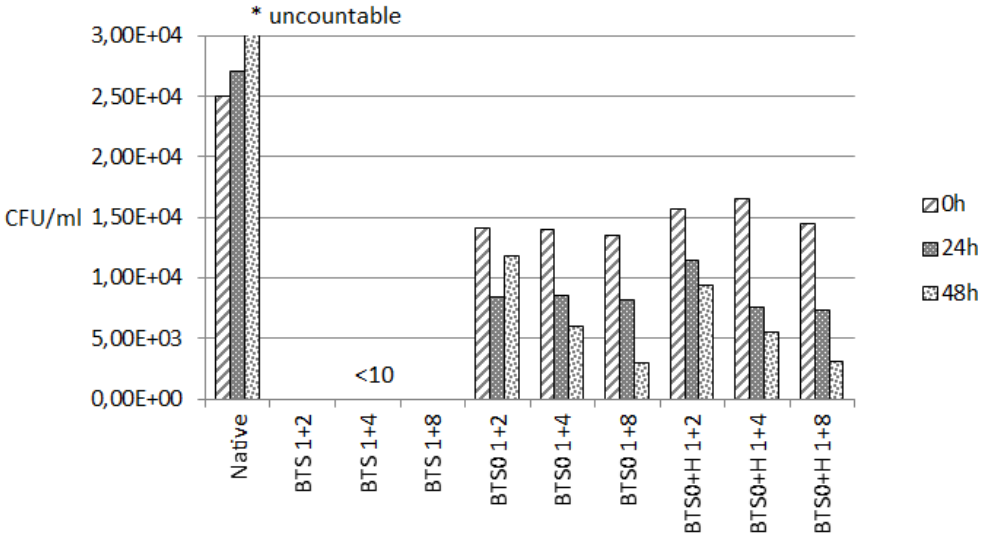


Figure 4. Determination of the colony-forming unit (CFU/ml) of microorganisms in extender BTS0 with added H and control samples (BTS, BTS0)



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