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Transformation of a short-term boar semen extender into a long-term boar semen extender by using penicillamine

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Abstract: This study aimed to convert a short-term semen extender into a long-term semen extender by adding penicillamine (PEN). Semen samples were collected from six boars, diluted at a concentration 3 × 10^9 spermatozoa/100 ml, and divided into eight groups as follows: BTS, Merck III®, Androstar® Plus, PEN 0.125 mM, PEN 0.25 mM, PEN 0.5 mM, PEN 1.0 mM, PEN 2.0 mM. All the diluted semen samples were stored at 18 °C and were examined for progressive motility, viability, acrosome integrity, pH and osmolarity on days 0, 1, 3, 5 and 7 after storage. The level of the total anti-oxidative capability (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were also measured for antioxidant activities. Fertility tests on a commercial pig farm was also conducted. On days 0, 1 and 3 of storage, no statistically significant differences in all the parameters among the extenders was observed. On day 5, the semen diluted with PEN at a concentration of 0.25 mM and Androstar® Plus could maintain progressive motility reach of 70.0% and 76.0%, respectively ($P < 0.01$). On day 7, the semen diluted with PEN at a concentration of 0.25 mM maintained progressive motility (70.0%) and acrosome integrity (85.7%) which is comparable to long-term semen extenders (Androstar® Plus) ($P < 0.01$). For the antioxidant activities, the level of the T-AOC, SOD and MDA varied depended on the PEN concentrations. The results of the fertility test were not different among the groups. To conclude, adding PEN at 0.25 mM in the BTS extender is able to transform a short-term semen extender into a long-term extender.

Keywords: antioxidant activity; artificial insemination; pig; reproduction; sperm

Artificial insemination (AI) is widely performed in the pig industry throughout the world (Gadea 2003). This technique is low cost, easily managed and diminishes the transmission of many venereal diseases on pig farms. One of the importance processes of AI includes a dilution of fresh boar semen in a semen extender. The commercially available semen extenders can be divided into three groups, e.g., short-term (less than or equal to three days), medium-term (less than or equal to five days) and long-term (less than or equal to seven days) extenders. After dilution, the diluted semen can be kept at 15–20 °C, which can reduce the sperm metabolic activity. Semen extenders provide the nutrients

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needed for the movement of sperm, controlling the osmotic pressure, pH buffering, the ions for the membrane balance and antibiotics to prevent microbial growth (Gadea 2003; Knox et al. 2008; Mapeka et al. 2012). In boars, the sperm plasma membrane consists of high levels of polyunsaturated fatty acids, which decrease dramatically when the spermatozoa are attacked by reactive oxygen species (ROS) due to lipid peroxidation during cold storage (Sikka et al. 1995). It has been elucidated that antioxidants not only have positive effects on the sperm qualities during cryopreservation (Chanapiwat and Kaeoket 2020), but also during cold storage, including decreasing the detrimental effect of ROS on the sperm plasma membrane leading to an increased sperm survival rate and acceptable sperm qualities after preservation (Funahashi and Sano 2005; Chanapiwat and Kaeoket 2021).

Penicillamine (PEN) is a derivative of penicillin; its D-isomer is a useful form (Wang et al. 2015). In human medicine, PEN is an anti-rheumatic drug for treating rheumatoid arthritis (Junker 1986), systemic sclerosis (Bluestone et al. 1970) and liver fibrosis (Kazemi et al. 2008) which can reduce the level of some heavy metal agents, such as copper (Cu), iron (Fe), lead (Pb), zinc (Zn) and many free radicals. Recently, it has been used as an antioxidant in the chilled storage of hamster, mouse, stallion and human semen in which the progressive motility of stallion semen can be maintained up to 50% after storage for eight days at room temperature (Aitken et al. 2012). In addition, it has also been reported that the supplementation of PEN in the freezing extender during cryopreservation of bull semen yielded superior progressive motility in the treatment than that of the control (Person et al. 2007; El-Shahat et al. 2017). Aitken et al. (2012) proposed that PEN may act as an antioxidant by binding with 4-HNE, the end-product of lipid peroxidation that enables the activate ROS generation, before they bind with the sperm protein, thereby neutralising their ability to disrupt motility. Consequently, PEN can significantly enhance the sperm motility and viability. It can be hypothesised that PEN, with its antioxidant activity as described by Aitken et al. (2012) , can be used as an antioxidant in a semen extender and may improve the quality of cold storage boar semen. However, no study has reported on the effects of PEN on the quality of cold storage boar semen. Therefore, the aim of this study was to investigate the optimal PEN concentration to add into a BTS based extender for storage of boar semen at 18 °C.

MATERIAL AND METHODS

This experiment was approved by the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC-Protocol No. MUVS-2015-09).

Animals

In total, six boars (two Pietrain boars, two Pietrain × Hampshire boars, two Pietrain × Duroc) that varied in age (between 2–4 years) were used. The boars were kept in an evaporative cooling system with individual pen. Each boar was used for the artificial insemination routine every 3–4 months and fed with a farm mixed diet twice daily with *ad libitum* water.

Semen collection

Semen was collected as earlier described by Chanapiwat and Kaeoket (2021). After collection, the volume, pH, sperm motility and concentration were evaluated. The ejaculates with a motility of more than 70% and morphology of more than 80% were used. The semen was diluted with each extender (3×10^9spermatozoa/100 ml) and stored in plastic artificial insemination bottles at a temperature of 18 °C for eight days and this semen was evaluated at 0, 1, 3, 5 and 7 days.

Extenders in different groups

Each semen sample was diluted with different extenders as described by Kaeoket et al. (2010a) and was further divided into eight groups as follows: the first three groups served as control commercial extenders consisting of BTS (short-term extender, Minitübe GmbH & CO. KG, Tiefenach, Germany), Merck-III® (medium-term extender, Minitübe GmbH & CO. KG, Tiefenach, Germany), Androstar® Plus (long-term extender, Minitübe GmbH & CO. KG, Tiefenach, Germany). The other five groups served as treatment groups which were diluted with the BTS extender, and supplemented with different

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PEN concentrations at 0.125 mM, 0.25 mM, 0.5 mM, 1 mM and 2 mM.

Sperm evaluation

All the semen samples were examined by the same examiner (Chanapiwat P.) who has more than 15 years' experience with semen evaluations, however, he was unaware of the treatment or control groups throughout the experiment period.

Sperm progressive motility

The percentage progressive motility of the spermatozoa was evaluated by Chanapiwat P. throughout experiment using a phase contrast microscope at 400× magnification (Chanapiwat et al. 2009).

Osmolarity and pH measurement

The pH parameter was measured by using a pH meter (ConsortC830; Consort, Turnhout, Belgium) whose range should be not lower than 6.5. The osmolarity of this semen was evaluated by using a single-sample osmometer (Fiske®Micro-Osmometer model 210; Advance Instrument Inc., Norwood, MA, USA) (Kaeoket et al. 2010a).

Sperm viability

The assessment of the sperm viability was evaluated by using SYBR-14/ethidiumhomodimer-1 (EthD-1). Ten (10) µl of diluted semen were mixed with 2.7 µl of the SYBR-14 solution and 10 µl of EthD-1 to estimate the viability after 15 min of incubation at 37 °C. Two hundred spermatozoa were evaluated using a fluorescence microscope at 400× magnification. The spermatozoa were separated into two types as live sperm (stained green) and dead sperm (stained red). The results were accordingly expressed by the percentage of live sperm (Kaeoket et al. 2012).

Sperm acrosome integrity

The diluted semen solution (10 µl) was mixed with 10 µl of EthD-1 and FitC-PNA, incubated at 37 °C for 15 min, 5 µl of semen was smeared on a glass slide and then fixed with 95% ethanol for 30 seconds. Then it was incubated at 4 °C in a moist chamber 30 min after spreading 50 µl of FitC-PNA (dilute FitC-PNA with PBS 1 : 10 v/v) over the slide (Chanapiwat et al. 2009; Kaeoket et al. 2010b). Thereafter, the slide was rinsed with a cold phosphate buffered solution (PBS) and air dried before the evaluation. Two hundred spermatozoa were evaluated using a fluorescent microscope at 1000× magnification, and classified either as an intact acrosome or a nonintact acrosome. The results were scored as the percentage of intact acrosomes of the spermatozoa.

Antioxidant activity analysis

The levels of the total anti-oxidative capability (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) on days 0, 3, 5, 7 after storage (semen collection day = day 0). The supernatant of the semen samples was collected by centrifugation at 2 000 rpm for 10 min, and kept at –20 °C until the analyses. The enzymatic activities of the T-AOC, and MDA were analysed with a spectrophotometric method using a T-AOC assay kit and an MDA kit (Biology Institute of Nanjing Jianchen, Jiangsu, China) (Hu et al. 2014) as follows in the next paragraphs.

Total anti-oxidative capability (T-AOC)

The T-AOC was measured using a T-AOC assay kit (Biology Institute of Nanjing Jianchen, Jiangsu, China) according to the manufacturer's instructions. The T-AOC activity was measured at 520 nm on a spectrophotometer (SPECTRONIC® 20 GENESYS™, Spectrum Chemical Mfg. Corp., New Brunswick, NJ, USA). The T-AOC activity of each sample was calculated into units per ml of supernatant (IU/ml).

Membrane lipid peroxidation

The Malondialdehyde (MDA) content (membrane lipid peroxidation product) was analysed with 2-thiobarbituric acid, monitoring the change of absorbance at 532 nm with a spectrophotom-

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eter (SPECTRONIC® 20 GENESYS™; Spectrum Chemical Mfg. Corp., New Brunswick, NJ, USA) by following the instructions of the MDA test kit (Biology Institute of Nanjing Jianchen, Jiangsu, China). The spermatozoa samples' MDA levels were expressed as nmol per ml of supernatant (nmol/ml).

Superoxide dismutase (SOD)

The SOD activity was measured using an SOD assay kit (Biology Institute of Nanjing Jianchen, Jiangsu, China) according to the manufacturer's instructions. The SOD activity was measured at 560 nm on a spectrophotometer (SPECTRONIC® 20 GENESYS™; Spectrum Chemical Mfg. Corp., New Brunswick, NJ, USA). The total SOD activity of each sample was calculated into units per ml of supernatant (IU/ml).

Glutathione peroxidase (GSH-Px)

The GSH-Px activity was determined using a GSH-Px assay kit (Biology Institute of Nanjing Jianchen, Jiangsu, China) by following the manufacturer's instructions. The GSH-Px activity was measured at 412 nm on a spectrophotometer (SPECTRONIC® 20 GENESYS™, Spectrum Chemical Mfg. Corp., New Brunswick, NJ, USA). The total GSH-Px activity of each sample was calculated into units per ml of supernatant (IU/ml).

Fertility tests on a pig farm

Standing oestrus detection, insemination, pregnancy diagnosis and fertility records. Oestrus detection was performed twice daily after weaning by examination of the vulva for its reddening and swelling along with controlling the standing reflex in the presence of a boar (Kaeoket et al. 2002; 2003). All the sows were inseminated twice with a conventional AI catheter at 12 h and 24 h after the standing oestrus with a dose of semen (boar of proven fertility), containing 3×10^9 spermatozoa in 100 ml of BTS (Group I, $n = 30$), Merck-III® (Group II, $n = 30$), Androstar® Plus (Group III, $n = 30$) and PEN-0.25 mM (Group IV, $n = 30$), kept at 18 °C for five days before insemination. The pregnancy test was performed on days 23–24 of pregnancy by transabdominal ultrasonography in the real-time B-mode (50STringa, sector probe with 5 MHz, ESAOTE Pie Medical, The Netherlands) (Kaeoket et al. 2005). The percentage of the pregnancy rate, the percentage of the farrowing rate, and the total number of piglets born were recorded.

Statistical analyses

All the sperm parameters were analysed by using the generalised linear model (GLM) (SPSS v22.0, SPSS Inc, Chicago, IL, USA) and expressed as mean ± SD. The experimental groups and the days of examination were modelled according to the factorial experiments in a completely randomised design (CRD). The effects of the groups on the parameters were analysed by using an analysis of variance (ANOVA) in the GLM. A P-value of less than 0.05 was considered as statistically significant.

RESULTS

The percentage of the progressive motility, viability and acrosome integrity on day 0, 1, 3, 5 and 7 are presented in Tables 1–5. The means of the osmolarity and pH are also presented in Tables 1–5. The semen qualities on days 0 and 1 were in a normal range (Tables 1 and 2). On day 3 (Table 3), comparing all the treatment groups, there were no statistically significant differences in all the parameters among the extenders.

On day 5 (Table 4), comparing all the treatment groups, the PEN 0.25 mM treated group had a significantly higher percentage of progressive motility (70.0%) than the other groups, but it was not different from the long-term extender (Androstar® Plus) ($P < 0.05$). On day 7 (Table 5), there were only two extenders that showed progressive motility of greater than 70%, i.e., PEN 0.25 mM (70.0%) and Androstar® Plus (73.0%), which had statistically significant differences from the other groups. These two extenders also had a higher percentage of acrosome integrity than the other groups (Androstar® Plus = 85.7% and penicillamine 0.25 mM treated group = 85.7%) ($P < 0.05$). For the percentage of viability, there was no significant difference among the groups (Tables 1–5).

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Table 1. The mean (± SD) percentage of the progressive motility, viability, acrosome integrity, osmolarity (mOsm/kg) and pH of the different extenders and at different penicillamine (PEN) concentrations on day 0 (n = 6)

Parameters	BTS	Merck-III®	Androstar® Plus	PEN 0.125	PEN 0.25	PEN 0.5	PEN 1.0	PEN 2.0
Progressive motility	72.0 ± 26.1	64.0 ± 25.3	76.0 ± 24.8	70.0 ± 19.0	70.0 ± 18.7	63.0 ± 23.4	65.0 ± 21.8	54.0 ± 21.9
Viability	76.9 ± 4.7	67.6 ± 9.3	72.0 ± 4.6	70.5 ± 10.2	75.4 ± 5.5	67.8 ± 14.5	70.6 ± 9.8	63.4 ± 12.7
Acrosome integrity	93.2 ± 2.3	89.2 ± 7.8	91.1 ± 2.9	91.1 ± 0.6	89.8 ± 3.4	92.4 ± 2.7	91.3 ± 1.7	92.3 ± 2.7
Osmolarity	323.0 ± 3.0	363.4 ± 15.2	302.8 ± 6.3	323.6 ± 4.5	336.6 ± 18.2	328.0 ± 8.0	327.0 ± 7.2	333.6 ± 15.6
pH	8.0 ± 0.2	8.0 ± 0.2	7.8 ± 0.2	7.8 ± 1.8	7.8 ± 0.1	7.8 ± 0.2	7.9 ± 0.1	7.9 ± 0.1

Table 2. The mean (± SD) percentage of the progressive motility, viability, acrosome integrity, osmolarity (mOsm/kg) and pH of the different extenders and at different penicillamine (PEN) concentrations on day 1 (n = 6)

Parameters	BTS	Merck-III®	Androstar® Plus	PEN 0.125	PEN 0.25	PEN 0.5	PEN 1.0	PEN 2.0
Progressive motility	73.0 ± 9.1	71.0 ± 19.2	84.0 ± 8.9	72.0 ± 18.2	73.0 ± 19.9	73.0±18.6	67.0 ± 7.6	64.0 ± 19.2
Viability	68.8 ± 10.8	63.9 ± 6.3	67.1 ± 9.4	71.1 ± 6.4	65.6 ± 6.7	62.5 ± 5.3	66.3 ± 4.0	65.7 ± 9.6
Acrosome integrity	91.0 ± 2.7	89.1 ± 4.1	87.0 ± 2.2	91.5 ± 3.7	92.5 ± 2.4	91.8 ± 2.2	90.2 ± 2.8	87.4 ± 3.3
Osmolarity	326.4 ± 4.4	360.4 ± 2.6	309.6 ± 7.8	326.4 ± 4.4	325.0 ± 8.0	321.0 ± 2.6	327.2 ± 4.3	324.6 ± 6.5
pH	8.0 ± 0.1	8.0 ± 0.2	7.9 ± 0.2	7.9 ± 0.1	7.9 ± 0.2	7.9 ± 0.1	7.9 ± 0.2	7.84 ± 0.2

Table 3. The mean (± SD) percentage of the progressive motility, viability, acrosome integrity, osmolarity (mOsm/kg) and pH of the different extenders and at different penicillamine (PEN) concentrations on day 3 (n = 6)

Parameters	BTS	Merck-III®	Androstar® Plus	PEN 0.125	PEN 0.25	PEN 0.5	PEN 1.0	PEN 2.0
Progressive motility	62.0 ± 11.5	55.0 ± 15.4	69.0 ± 14.3	69.0 ± 12.4	68.0 ± 13.0	67.0 ± 14.4	63.0 ± 12.0	56.0 ± 15.6
Viability	76.2 ± 8.5	70.7 ± 6.0	74.6 ± 9.2	74.1 ± 8.4	70.8 ± 6.8	70.2 ± 5.0	70.9 ± 4.6	72.5 ± 9.1
Acrosome integrity	89.3 ± 2.0	89.3 ± 4.2	91.0 ± 3.7	90.5 ± 2.7	90.6 ± 2.2	92.2 ± 2.1	89.8 ± 2.4	88.2 ± 4.2
Osmolarity	323.8 ± 1.9	358.0 ± 7.5	303.0 ± 7.8	320.0 ± 5.8	318.0 ± 3.5	320.6 ± 5.7	321.0 ± 2.6	324.6 ± 1.8
pH	7.8 ± 0.2	8.1 ± 0.2	7.8 ± 0.2	7.8 ± 0.1	7.9 ± 0.2	7.9 ± 0.1	7.9 ± 0.1	7.8 ± 0.2

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Table 4. The mean (± SD) percentage of the progressive motility, viability, acrosome integrity, osmolarity (mOsm/kg) and pH of the different extenders and at different penicillamine (PEN) concentrations on day 5 (n = 6)

Parameters	BTS	Merck-III®	Androstar® Plus	PEN 0.125	PEN 0.25	PEN 0.5	PEN 1.0	PEN 2.0
Progressive motility	53.0 ± 11.5^{c}	47.0 ± 12.0^{c}	76.0 ± 4.2^{a}	63.0 ± 14.8^{abc}	70.0 ± 12.2^{ab}	63.0 ± 11.0^{abc}	59.0 ± 8.9^{bc}	48.0 ± 14.8^{c}
Viability	74.3 ± 6.6	71.6 ± 8.5	75.5 ± 4.8	73.0 ± 7.7	76.5 ± 8.8	72.1 ± 8.2	69.0 ± 5.5	69.6 ± 4.9
Acrosome integrity	87.3 ± 4.7	88.4 ± 5.1	90.3 ± 2.9	89.25 ± 1.4	88.8 ± 3.6	89.0 ± 8.8	88.8 ± 2.0	87.5 ± 2.9
Osmolarity	323.8 ± 3.9	358.0 ± 4.2	302.0 ± 3.1	329.8 ± 5.4	322.4 ± 1.8	322.8 ± 3.4	323.8 ± 3.6	323.0 ± 2.6
pH	7.9 ± 0.2	8.0 ± 0.1	7.73 ± 0.1	7.86 ± 0.1	7.8 ± 0.2	7.7 ± 0.4	7.8 ± 0.2	7.7 ± 0.2

$^{a–c}$Values followed by different letters within the same row against each parameter were significantly different (P < 0.01)

Table 5. The mean (± SD) percentage of the progressive motility, viability, acrosome integrity, osmolarity (mOsm/kg) and pH of the different extenders and at different penicillamine (PEN) concentrations on day 7 (n = 6)

Parameters	BTS	Merck-III®	Androstar® Plus	PEN 0.125	PEN 0.25	PEN 0.5	PEN 1.0	PEN 2.0
Progressive motility	46.0 ± 8.9^{bc}	44.0 ± 16.7^{c}	73.0 ± 6.7^{a}	63.0 ± 14.4^{ab}	70.0 ± 13.7^{a}	57.0 ± 14.8^{abc}	50.0 ± 10.6^{bc}	49.0 ± 12.9^{bc}
Viability	72.1 ± 5.1	69.5 ± 3.0	73.1 ± 5.1	68.3 ± 6.5	68.6 ± 7.5	67.3 ± 6.2	64.2 ± 5.5	61.3 ± 7.4
Acrosome integrity	78.7 ± 2.9^{c}	78.9 ± 2.3^{c}	85.7 ± 2.0^{a}	81.6 ± 3.0^{bc}	85.7 ± 2.9^{a}	81.6 ± 3.3^{bc}	83.7 ± 2.4^{ab}	78.8 ± 2.3^{c}
Osmolarity	321.8 ± 1.9	356.0 ± 3.5	297.2 ± 0.5	320.4 ± 5.4	318.6 ± 4.0	322.8 ± 4.6	321.2 ± 5.0	325.0 ± 5.3
pH	7.9 ± 0.1	7.8 ± 0.1	7.62 ± 0.2	7.7 ± 0.1	7.7 ± 0.2	7.72 ± 0.2	7.7 ± 0.1	7.73 ± 0.2

$^{a–c}$Values followed by different letters within the same row against each parameter were significantly different (P < 0.01)

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Due to the T-AOC, SOD, GSH-Px and MDA levels, five boars were under detectable limit throughout the experimental period. Therefore, only the completed result of one boar on day 7 is presented (Figure 1).

For the fertility tests on the pig farm after preservation, the extended fresh boar semen in each semen extender for 5 days at 18 °C, the pregnancy rate, the farrowing rate and the total number of piglets born are presented in Table 6 ($P > 0.05$). In group IV, a BTS-based extender, contained PEN at a concentration of 0.25 mM, showed fertility results comparable to those of commercial boar semen extenders.

Table 6. The percentage of the pregnancy rate (PR), farrowing rate (FR), total number of piglets born (TB) of the different groups ($n = 30$)

Groups	%PR	%FR	TB (means ± SD)
I	96.7	83.3	13.36 ± 2.48
II	93.7	84.4	12.68 ± 2.50
III	90.0	76.7	12.43 ± 2.19
IV	93.3	86.7	11.58 ± 3.19
Statistical analysis	NS	NS	NS

Group I = BTS; Group II = Merck-III®; Group III = Androstar® Plus; Group IV = adding PEN at 0.25 mM; NS = not significant

An average %FR of this farm during the experimental period is 83%

DISCUSSION

In the present study, we compared the semen parameters, such as progressive motility, viability and acrosome integrity of boar sperm, diluted in different extenders during storage at 18 °C for seven days, and also conducted fertility tests on a commercial pig farm. The present results showed that the supplement of penicillamine at a concentration of 0.25 mM could maintain the sperm motility, acrosome integrity up to seven days and also its fertility test after cold storage for five days on a commercial pig farm which is comparable to a long-term extender (i.e., Androstar® Plus). This agrees with the earlier results reported by Aitken and Curry (2011) in that PEN maintained progressive motility of stallion semen up to 50% after storage for eight days at room temperature. Rationally, BTS and Merck-III® preserved the semen quality up to three days as claimed by a manufacturer. It has long been known that excessive ROS formation by sperm metabolism during cold storage has been related to the destruction of the plasma membrane, and subsequently decreasing the sperm motility (De Lamirande et al. 1997; Guthrie et al. 2002; Kumaresan et al. 2009). In addition, ROS induce lipid peroxidation is related to the generation

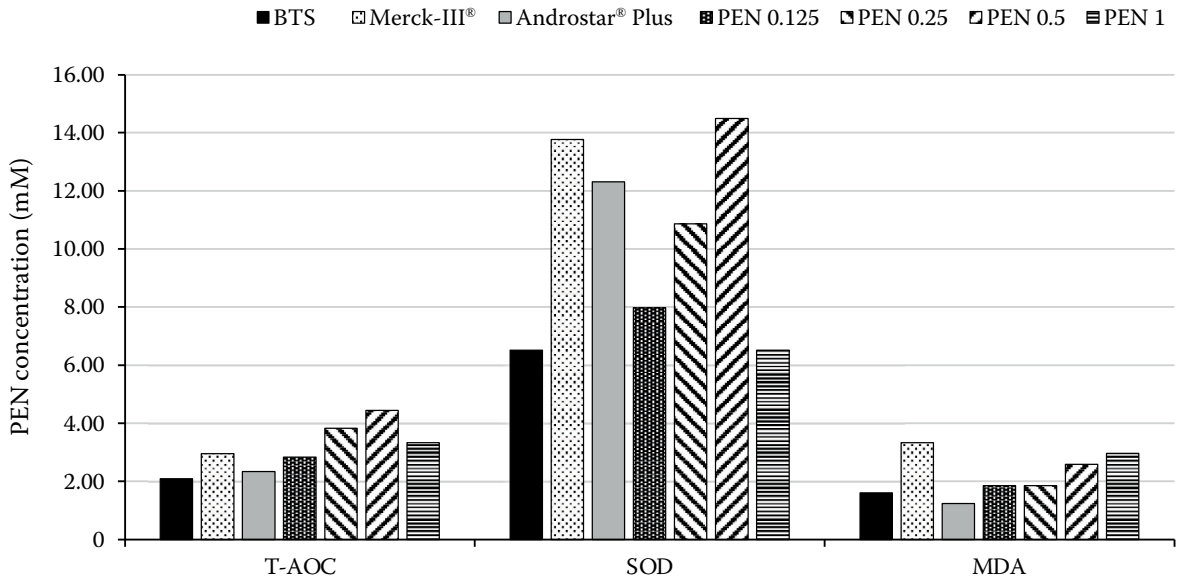

Figure 1. The T-AOC (IU/ml), superoxide dismutase (SOD) (IU/ml) and malondialdehyde (MDA) (nmol/ml) levels in the different extenders and at different penicillamine (PEN) concentrations on day 7 of storage at 18 °C

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of electrophilic cytotoxic aldehydes, such as 4-hydroxynonenal (4-HNE) (Jones et al. 1979; Diezel et al. 1980; Aitken et al. 2012). Electrophilic cytotoxic aldehydes are binding protein thiols that regulate the sperm movement so the sperm are less motile and cause the positive feedback in the ROS generation (Alvarez and Storey 1995; Aitken and Curry 2011). Aitken et al. (2012) proposed that PEN may act as an antioxidant by binding with 4-HNE, the end product of lipid peroxidation that enables the ROS generation to activate, before they bind with the sperm protein, thereby neutralising the ability to disrupt the motility. Besides, the levels of T-AOC, SOD and MDA on day 7 in the present results indicated that the antioxidant capacity (i.e., T-AOC and SOD) and lipid peroxidation (i.e., MDA) are dependent on the PEN concentration in each group of extenders (Hu et al. 2014). It is worth noticing that BTS, a short term-extender (i.e., without an antioxidant and lipid peroxidation inhibitor) in which semen can only be stored for three days could not diminish the oxidation and lipid peroxidation during cold storage. This underlining mechanism may explain the significantly enhanced boar sperm progressive motility (70%) and viability (85.7%) during cold storage at 18 °C for seven days in the present study. This is in agreement with our recent study (Chanapiwat and Kaeoket 2021) in that a high potential antioxidant, namely L-cysteine, can be added into a short-term semen extender in order to preserve the fresh boar semen quality for as long as seven days after collection for the long distance transportation and also in accordance with our previous study in cryopreserved boar semen (Kaeoket et al. 2010c) in that the L-cysteine supplementation at the optimal concentration of 5 mM or 10 mM improved the post-thawed boar semen qualities. In practice, this also implied that pig farmers or boar stations can pay a lower cost for a long-term semen extender if they supplement a high potential antioxidant (i.e., PEN) into the existing short-term semen extender at their farm or boar station without any undesirable effect on the farm fertility.

CONCLUSION

Taking all the results together, it can be concluded that the supplementation of 0.25 mM penicillamine in a BTS-based extender could maintain the sperm progressive motility and acrosome integrity of fresh extended boar semen to ensure that it is comparable to those of long-term semen extenders.

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Conflict of interest

The authors declare no conflict of interest.

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