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Effects of Catechin Addition to Extender on Sperm Quality and Lipid Peroxidation in Boar Semen

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Abstract: The purpose of this study was to investigate the effects of catechin in boar semen with extender *in vitro*. Freshly ejaculated boar semen was diluted with Beltsville Thawing Solution (BTS) and supplemented with 5 levels of catechin (0, 25, 50, 75 and 100 μM). Sperm quality (motility, viability and acrosome integrity) and lipid peroxidation (malondialdehyde) were evaluated at 24, 48 and 72 hrs. after incubation. The results revealed that the motility of boar sperm supplemented with catechin was higher than that of the control group ($P<0.05$). In addition, after incubating the sperm for 24 and 72 hrs., sperm viability of the groups supplemented with catechin were higher than that of the control group ($P<0.05$). Besides, acrosome integrity of boar sperm in the groups supplemented with catechin were lower than that in the control group ($P<0.05$). At 24, 48 and 72 hrs. of the experimental period, malondialdehyde of the groups with added catechin were lower than that of the control group ($P<0.05$). It can be concluded that catechin could improve sperm quality and reduce lipid peroxidation in boar semen.

Key words: Catechin · Boar · Lipid peroxidation · Sperm quality · Semen

INTRODUCTION

Oxidative stress is known to play a major role in the aetiology of defective sperm function via mechanisms involving the induction of peroxidative damage to the plasma membrane [1,2], a subsequent reduction of sperm motility [2,3] and a decline in cell quality which results in insufficient numbers of viable spermatozoa and fertility [2]. Like all cells living under aerobic conditions, spermatozoa produce reactive oxygen species (ROS), mostly originating from normal metabolic activity. Spermatozoa generates the superoxide anion ($^{\circ}O_2^-$) which spontaneously or enzymatically dismutases to hydrogen peroxide (H_2O_2). H_2O_2 is relatively stable and has a higher oxidant potential [4]. The first report that ROS could have harmful effects on sperm has been published for over 60 years [5]. ROS is believed to be one of the most important mediators of damage to spermatozoa that have been associated with various indices of cellular injury. Lipid peroxidation has been correlated with exposure of sperm to ROS [6-8]. In addition, excessive ROS formation by spermatozoa has been associated with decreased motility and abnormal morphology [9]. Tremellen [10] reported the effect of ROS to sperm that ROS first damages the sperm membrane which in turn reduces the sperm's motility and ability to fuse with the oocyte and then directly damages sperm DNA, compromising the paternal genomic contribution to the embryo. Generally, sperm membranes are particularly susceptible to ROS damage because of their high content of polyunsaturated fatty acid and their lack of defensive mechanisms [11].

Catechins are another group of polyphenolic flavonol compound derived from green tea [12]. Catechin concentrations are especially high in broad beans, black grapes, apricots and straw berries [13]. Catechin has potentially positive effects on human health as suggested by *in vitro* studies [14]. Studies in humans have suggested that catechin increased plasma antioxidant activity and decreased plasma lipid peroxide and malondialdehyde concentration [13]. Moreover, dietary supplementation with catechin reduces lipid peroxidation in pig meat [12]. Whereas, data about the effect of

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catechin on sperm quality in boar have been limited, we have hypothesized that catechin could reduce the effects of lipid peroxidation and improve sperm quality of boar during incubation. However, the aim of this experiment was to study the effects of catechin in terms of incubation time on sperm abnormality and sperm quality and lipid peroxidation in boar semen. The results from this study would provide fundamental knowledge of using antioxidants for improving sperm quality in boar semen and benefit of artificial insemination in the swine production.

MATERIALS AND METHODS

Semen Collection: One ejaculate from 2-to-3yr-old purebred boar of the Duroc Jersey (individual penned in 2x2.5-m pen) was collected at the Artificial Insemination Unit of Swine Section, Mahasarakham University Farm using the gloved-hand technique [15, 16]. At collection, the sperm-rich fraction of the ejaculate was filtered through sterile gauze to remove gelatinous material [17,18]. Immediately after collection, general semen quality such as volume, opacity, color, odor and turbidity were assessed before further processes. Then, the semen was transfered to the laboratory of the Faculty of Veterinary Medicine and Animal Science, Mahasarakham University and was kept in a box at room temperature.

Preparation of Sperm Suspension: After the semen arrived in the laboratory (60 min. after collection), it was fractionated visually into sperm-rich and sperm-poor portions by using opacity, an indication of the number of sperm cells and only sperm-rich portion was used. The sperm concentration was determined by a hemocytometer and was kept at 25 degree C. Semen was dispensed in $3x10^9$ sperm aliquots into insemination bottle with 50 ml of Beltsville thawing solution (BTS).

Experimental Design: This experiment was designed to evaluate the effect of catechin on sperm quality. The concentration of catechin in suspension used in this experiment was divided into 5 treatments (T) i.e. T1 (0 μM, control group), T2 (25 μM), T3 (50 μM), T4 (75 μM) and T5 (100 μM). The suspension of semen was incubated in the controlled refrigerator at 18 °C. Evaluation of semen was performed at 24, 48 and 72 hrs. Catechin used in this experiment was (+)-Catechin hydrate, a product of Fluka®, Lot 1288041 and Filling code 44206220.

Evaluation of Semen

Sperm Viability: Sperm viability was assessed by an eosin exclusion test. Five microliters of sperm dilution were mixed with 5 microliters of eosin solution. Immediate counting of fraction of uncolored cells was used to calculate the percentage sperm viability [15]. Two hundred spermatozoa were evaluated to determine viability [16].

Sperm Motility: Progressive motility of sperm was evaluated by light microscopy under 400x magnifications with a thermal stage at 37°C for 3 times by the same observer. Two hundred spermatozoa were evaluated to determine motility [16].

Acrosome Integrity: One drop of each sample was added with five drops of eosin-nigrosin, mixed together and then stored under room temperature for 60 seconds. The slide was immediately dried by using a hot plate. The percentage of acrosome abnormality was determined by counting a total of 200 spermatozoa under light microscopy (magnification 100x, oil immersion). Spermatozoa were classified into three categories: intact acrosome, damaged acrosome and missing acrosome [19].

Malondialdehyde Concentrations: The concentration of malondialdehyde (MDA), an index of lipid peroxidation (LPO) in sperm samples, was measured in accordance with the method described by Placer *et al.* [20]. The values of MDA were expressed as μM.

Statistical Analysis: The dependent variables (percentage of sperm motility, viability, acrosome integrity and malondialdehye concentration) were evaluated by using ANOVA and Duncan's Multiple Range Test (DMRT) (1955) to locate differences. Values were presented as mean±S.D. and the level of significance was set at $P < 0.05$.

RESULTS

The Results of the Experiment Can Be Shown as Follows

Sperm Motility: At 24 hrs of the incubation, the sperm motility of T2 (25 μM) and T3 (50 μM) were significantly higher than that of T1 (0 μM) and T5 (100 μM) ($P<0.05$). At 48 hrs, the sperm motility of T2, T3 and T4 (75 μM) were significantly higher than that of T1 and T5 ($P<0.05$). At 72 hrs, the sperm motility of T3 was significantly higher ($P<0.05$) than that of T1, T2, T4 and T5 (Table 1).

Table 1: Effects of catechin addition to extender of boar semen on sperm motility (%) at 24, 48 and 72 hrs of incubation period

	Catechin levels				
Experimental period	T1 (Control)(n=9)	T2 (25 μM)(n=9)	T3 (50 μM)(n=9)	T4 (75 μM)(n=9)	T5 (100 μM)(n=9)
24 h	79.66 ± 2.73^{c}	89.11 ± 2.70^{a}	88.55 ± 2.14^{a}	85.88 ± 3.53^{ab}	80.87 ± 3.37^{bc}
48 h	77.44 ± 0.84^{b}	83.55 ± 1.07^{a}	84.00 ± 1.20^{a}	83.66 ± 4.91^{a}	75.22 ± 2.17^{b}
72 h	69.11± 1.57^{b}	71.44±3.29^{b}	76.22 ± 1.34^{a}	72.55 ± 0.96^{b}	71.89 ± 0.84^{b}

Within a row, means without a common superscript (a-c) differed ($P < 0.05$)

Table 2: Effects of catechin addition to extender of boar semen on sperm viability (%) at 24, 48 and 72 hrs of incubation period

	Catechin levels				
Experimental period	T1 (Control)(n=9)	T2 (25 μM)(n=9)	T3 (50 μM)(n=9)	T4 (75 μM)(n=9)	T5 (100 μM)(n=9)
24 h	90.00 ± 2.65^{b}	91.11 ± 2.59^{b}	93.22 ± 0.51^{ab}	91.86 ±1.22^{ab}	94.89 ±0.77^{a}
48 h	86.89 ± 2.14	87.33 ± 1.21	90.22 ± 0.51	89.45 ± 2.34	89.11 ± 2.53
72 h	80.00 ± 2.65^{b}	85.67 ± 2.52^{a}	87.00 ± 1.86^{a}	87.33 ± 2.00^{a}	86.00 ± 2.41^{a}

Within a row, means without a common superscript (a-b) differed ($P < 0.05$)

Table 3: Effects of catechin addition to extender of boar semen on acrosome integrity (%) at 24, 48 and 72 hrs of incubation period

	Catechin levels				
Experimental period	T1 (Control)(n=9)	T2 (25 μM)(n=9)	T3 (50 μM)(n=9)	T4 (75 μM)(n=9)	T5 (100 μM)(n=9)
24 h	8.33 ± 1.21^{ab}	9.44 ± 1.07^{a}	6.00 ± 1.86^{bc}	5.00 ± 1.73^{c}	6.56 ± 1.64^{abc}
48 h	9.33 ± 0.34^{a}	6.78 ± 0.51^{b}	5.44 ± 0.20^{c}	4.22 ± 0.19^{d}	4.67 ± 0.67^{d}
72 h	11.22 ± 0.96^{a}	4.67 ± 0.58^{b}	3.78 ± 0.51^{bc}	2.56 ± 1.17^{cd}	2.00 ± 0.33^{d}

Within a row, means without a common superscript (a-d) differed ($P < 0.05$)

Table 4: Effects of catechin addition to extender of boar semen on malondialdehyde concentration (nM/3x10^{7}cell) at 24, 48 and 72 hrs of incubation period

	Catechin levels				
Experimental period	T1 (Control)(n=9)	T2 (25 μM)(n=9)	T3 (50 μM)(n=9)	T4 (75 μM)(n=9)	T5 (100 μM)(n=9)
24 h	59.67 ± 4.34^{a}	41.13 ± 8.35^{b}	33.27 ± 4.31^{b}	35.23 ± 5.97^{b}	31.90 ± 6.93^{b}
48 h	39.88 ± 3.48^{a}	34.97 ± 2.12^{ab}	33.32 ± 1.95^{b}	25.87 ± 3.33^{c}	25.23 ± 4.94^{c}
72 h	34.59 ± 0.97^{a}	28.95 ± 3.90^{b}	26.77 ± 1.78^{bc}	23.30 ± 0.80^{cd}	22.15 ± 0.44^{d}

Within a row, means without a common superscript (a-d) differed ($P < 0.05$)

Sperm Viability: After the incubation for 24 hrs, the sperm viability of T5 was significantly higher than that of T1 and T2 ($P<0.05$). At 48 hrs, the sperm viability of T1 to T5 were not significantly different between the treatments ($P>0.05$). At 72 hrs, the sperm viability of T2 to T5 were significantly higher ($P<0.05$) than that of T1 (Table 2).

Acrosome Integrity: At 24 hrs of the incubation, the acrosome integrity of T4 was significantly lower than that of T1 and T2 ($P < 0.05$), but not significantly different from that of T3 and T5 ($P > 0.05$). At 48 hrs, the acrosome integrity of T4 and T5 were significantly lower than that of T1, T2 and T3 ($P < 0.05$) and the acrosome integrity of T3 was significantly lower than that of T1 and T2 ($P < 0.05$). Moreover, the acrosome integrity of T2 was significantly lower than that of T1 ($P < 0.05$). At 72 hrs, the acrosome integrity of T5 was significantly lower than that of T1, T2 and T3 ($P < 0.05$) and the acrosome integrity of T2 and T3 were significantly lower ($P < 0.05$) than that of T1 (Table 3).

Malondialdehyde Concentration: At 24 hrs of the incubation, the malondialdehyde concentration of T2, T3, T4 and T5 were significantly lower than that of T1 ($P<0.05$). At 48 hrs, the malondialdehyde concentration of T4 and T5 were significantly lower than T1, T2 and T3 ($P<0.05$). At 72 hrs, the malondialdehyde concentration of T2, T3, T4 and T5 were significantly lower than that of T1 ($P<0.05$) and the malondialdehyde concentration of T4 and T5 were significantly ($P<0.05$) lower than that of T2 (Table 4).

DISCUSSION

With different levels of catechin in extended boar sperm, the progressive sperm motility, sperm viability, acrosome integrity and malondialdehyde levels were determined at 24, 48 and 72 hrs of the incubation periods. The results showed that at 24 and 48 hrs, the motility of T2 and T3 were higher than that of T1 and at 72 hrs, the motility of T3 was higher than those of the other groups. These results indicated that T3 could improve the boar sperm motility after all incubation periods. This is in accordance with the findings of Thuwanut *et al.* [21] and Pena *et al.* [22]. They supplemented antioxidants i.e. DL-cystein and vitamin E analogue; and vitamin E analogue Trolox in an extender and found that the antioxidants could improve the motility of cat frozen-thawed epididymal sperm and boar sperm, respectively. In addition, Michael *et al.* [23] studied the effect of antioxidant on the motility of canine sperm and found that catalase, NAC (N-acetyl-cysteine) and vitamin E could improve the motility and rapid steady forward movement (RSF movement) of post-thaw canine sperm, *in vivo*. Besides, Marin-Guzman *et al.* [24] supplemented selenium in the diet of a boar and found that the motility of the boar sperm increased. Moreover, Bucak *et al.* [25] supplemented trehalose, taurine and cysteamine in an extender of ram semen and found that this could also increase the sperm motility.

After 24 hrs of incubation, the sperm viability of T5 was higher than that of T1 and at 72 hrs, the sperm viability of T2 to T5 were higher than that of T1. This result showed that catechin could improve boar sperm viability during incubation period too. The result from this study is in accordance with the report of Cerolini *et al.* [26], which states that the addition of alpha-tocopherol in the semen extender could help improve the viability of boar sperm.

At 24 hrs, the acrosome integrity of T3, T4 and T5 were lower than that of T1. At 48 hrs, the acrosome integrity of T2 to T5 were lower than that of T1. Moreover, at 72 hrs, the acrosome integrity of T5 was lower than those of T1, T2 and T3. These results indicated that T5 could reduce the acrosome integrity of boar sperm. This is in accordance with the report of Gadae *et al.* [27] who conclude that reduced glutathione could reduce acrosome integrity, improving the function and fertilizing capacity of frozen boar spermatozoa.

At 24, 48 and 72 hrs of the incubation period, the malondialdehyde of T3, T4 and T5 were lower than that of T1. This is similar to the report of Brzezińska-Ślebodzińska *et al.* [28] which finds that vitamin E reduced glutathione against fatty acid peroxidation in boar semen. Moreover, Cerolini *et al.* [26] found that alpha-tocopherol in semen extender could help reduce the malondialdehyde levels in the extender of boar semen. On the other hand, these results are different from the report of Bucak *et al.* [25] who found that trehalose, taurine, cysteamine and hyaluronan, supplemented in the extender, did not cause any differences in the levels of malondialdehyde in ram semen after the freeze-thawing process.

Generally, safety in the use of catechine was approved in both human and animals. When comparing the effectiveness of catechin in this study with other substances in the previous reports, we found that vitamin E [16,21,22,28], DL-cysteine [29], N-acetyl-cysteine (NAC) [23], trehalose, taurine, cysteamine [25], 1-*O*-Alkylglycerols [15] and reduced glutathione [27] could improve only some parameters of sperm quality. For example, vitamin E, trehalose, taurine, cysteamine and 1-O-Alkylglycerols could improve only sperm motility and alpha-tocopherol could increase sperm viability and reduce malondialdehyde level. By comparing the effectiveness of catechin in this study with butylated hydroxytoluene we found that both substance could increase sperm motility; reduce viability, acrosome integrity and malondialdehyde level. However, butylated hydroxytoluene was studied against cryopreservation injuries to boar spermatozoa and improved sperm quality after thawing, but catechin was studied for the benefit of preserving boar semen with extender. Lastly, in this study catechin in extender at 50 μM could prevent oxidative damage, improve sperm motility and viability and reduce acrosome integrity during storage.

CONCLUSION

Addition of catechin in an extender with boar spermatozoa could improve sperm motility, viability, acrosome integrity and reduced malondialdehyde level during the incubation periods. The antioxidant property of catechin could improve sperm quality better than vitamin E, DL-cysteine, N-acetyl-cysteine (NAC), trehalose, taurine, cysteamine and reduced glutathione etc. Therefore, catechin may be used as an antioxidant for reducing sperm abnormality and improving sperm quality in boar semen.

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