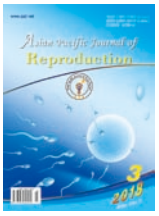

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Effect of different concentration of fish oil in skim milk-egg yolk extenders on post-thawed semen qualities of Kalang swamp buffalo bull

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ABSTRACT

Objective: To explore the effect of fish oil at different concentrations on post-thawed semen of Kalang swamp buffalo. **Methods:** A total of 4 Kalang swamp buffalo bulls with 3-5 years of age and weighed about 340-360 kg were slected. Semen was regularly collected from these buffalo bulls once a week by an artificial vagina. Fish oil was supplementary at the dosages of 0 mg (control), 50 mg, 100 mg, 150 mg, and 200 mg to the extender (skim milk-egg yolk). Fresh, pre-freezing and frozen semen were thawed at 37 ℃ and evaluated for motility, viability, morphology, and plasma integrity of membrane. **Results:** The study results indicated that before freezing, supplementation of fish oil at the dose of 150 mg in the extender had significantly motility. And a significant (P<0.05) increase was observed in viability and motility of post-thawed semen at the dose of 150 mg fish oil, which was in difference with other treatment groups. **Conclusions:** Addition of 150 mg fish oil in the extender could be positive for the enhancement of the quality of post-thawed semen of Kalang swamp buffaloes.

1. Introduction

Kalang swamp buffalo is a species of swamp buffalo, a native from Amuntai district, South Kalimantan province, Indonesia. Its pattern of maintenance differs from other types of swamp buffalo in the world. Everyday early in the morning, the buffalo go out of the cage (Kalang) to soak in the swamp for a day and then afternoon climb into the cage for rest. The population of Kalang swamp buffalo during five years has decreased, it is due to the number of cuts that is unbalanced with the development of the population and the low fertility[1]. One of the important factors to increase kalang swamp buffalo population is a estrus synchronization program trailed by artificial insemination (AI), because Kalang buffalo has unclear symptoms of estrus. While, an significant factor that determines the success of AI is the qualities of post-thawing semen that will affect conception and percentage of pregnancy[2].

Application of AI program in swamp buffaloes has been reported, but the results are not as good as in the application of AI in cows. The main cause of low AI success in swamp buffaloes is due to low quality of post-thawed sperm and low fertility level of female buffalo[3-7]. Furthermore, Andrabi[8] reported that there were many aspects that could affect the sperm motility, integrity of membrane and sperm viability through storage including cryopreservation and thawing process.

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The freezing process of semen needs a step-by-step process that begins with microscopic and macroscopic semen evaluation, dilution, cooling, equilibration and freezing weakens the naturally existing seminal antioxidant capacity. Consequently, many strategies have been employed to progress the efficiency of cryopreservation process. However, the overall success to increase the effectiveness of the cryopreservation of buffalo semen remains relative low. In this condition, the exogenous antioxidants addition in extender has provided a great opportunity to increase sperm quality to struggle the oxidative stress during cryopreservation[9].

One of the ingredients that can be used to help increase the sperm quality of buffalo post-thawing is fish oil. Fish oil contains several active ingredients such as omega 3 which is the main source of eicosapentaenoic acid, docosahexaenoic acid, saturated fatty acid and polyunsaturated fatty acids (PUFA)[10,11]. Several researches have been done on the effects of using fish oil to increase the semen quality either through feed or mixed with diluents in domestic animals. Meanwhile, the use of fish oil containing saturated fatty acids has been stated to be used to increase spermatozoa survival in general, membrane integrity and motility in boar[12] and cattle[13,14].

However, the influence of fish oil on Kalang buffalo spermatozoa has never been conducted. Therefore, the objective of the present study was carried out to determine the influence of fish oil supplementation in the extender on the sperm motility, viability, abnormality and integrity of membrane of cryopreserved buffalo bull sperm.

2. Materials and methods

The study was conducted at the center of artificial insemination, province of South Kalimantan at Banjarbaru, Indonesia. The approval of the animal ethics committee on the conduct of this study was not necessary because there no invasive techniques on animals. Semen was used in the process of collection, evaluation and freezing by using standard method under the central artificial insemination program.

A total of 4 Kalang swamp buffalo bulls aged 3-5 years and weighed about 340-360 kg were used in the study. All bulls were kept in individual cage with the same feed. Forage was given to livestock about 10% of body weight of buffalo bull. Concentrate containing approximately 16% crude protein and 2.6% crude fat was given 3 kg/buffalo/day to each buffalo bull and water ad libitum. Semen was regularly collected from all bulls once a week by an artificial vagina. A total of 24 samples were poised and then transferred at 37 ℃ in water bath to the center of artificial insemination of Banjarbaru for evaluation.

2.1. Semen handling

The extender base used comprises of skim milk and egg yolk, containing 10% skim mlik, 5% (v/v) egg yolk, 1% (w/v) fructose (Scharlau, Barcelona, Spain), 8% (v/v) glycerol (Merck, Darmstadt, Germany), penicillin (1 000 IU/mL) and streptomycin (1 000 mg/mL). The samples were designed to compare the effect of different concentrations of fish oil (liquid) (Manufacture Pty, Ltd. Minna Close Belrose, Australia) with the dose of 0 mg (control), 50 mg, 100 mg, 150 mg or 200 mg in the 100 mL skim milk-egg yolk extender. Semen was diluted with final sperm concentration 25×10^6/mL. Diluted semen was gradually cooled to 4 ℃ for 2 h. After equilibration, cooled semen was wrapped in 0.5 mL French straws, then placed on top of liquid nitrogen vapor (about 5 cm above liquid nitrogen) for 10 min. The straws were then dipped continuously and stored into liquid nitrogen. Later one week of kept in liquid nitrogen, five semen straws from each treatment group in each replicate were melted at 37 ℃ for 30 s and analyzed for the following parameters.

2.2. Evaluation of sperm qualities

Motility, viability, and abnormality parameters was measured on fresh semen and post-thawing semen. Motility of sperm was evaluated when a small droplet (10 μL) of fresh, pre-freezing sperm and post-thawing sperm was placed in the center of a pre-warmed slide and then covered with cover slip, and observed under phase contrast microscope at 400× magnification. Morphology of sperm and viability were observed by using eosin-nigrosin stain as adopted by Khumran *et al*[15]. At least of 200 sperms were counted in four microscopic diverse fields adopted by Memon *et al*[16]. Morpholog of sperm was observed using the same slide used for sperm viability. The normal of sperm cells was counted from a total sperm cells examined. The sperm integrity of membrane was assessed in fresh, pre-freezing semen, and post-thawed semen. Integrity of membrane was evaluated using hypo osmotic swelling test as defined by Kaka *at al*[17].

2.3. Statistical analysis

The effects of difference concentration of fish oil in the extender on motility, viability, integrity of membrane, and abnormality were analyzed by one-way analysis of variance (ANOVA). The collected data were presented as mean ±SD, and were significant at $P<0.05$.

3. Results

Observation of fresh semen by macroscopic and microscopic evaluation showed the following results: the average volume was (2.90±0.09) mL, pH was 6.80±0.15 and semen concentration was 1.5×10^6/mL. The average percentages of fresh semen were (71.00±1.04)%, (78.00±0.81)%, (15.00±1.02)% and (73.00±0.93)% for sperm motility, viability, abnormality and integrity of membrane,

respectively. The sperm motility of Kalang swamp buffalo bull semen stored for 4 h before freezing at dose of 150 mg fish oil was higher (P<0.05) than those at dose of 50 mg and 100 mg fish oil; however, the treatment with 200 mg fish oil caused a significant (P<0.05) reduction in motility (Table 1).

Significant (P<0.05) increases were detected in semen motility and viability of post frozen-thawed sperm at dose of 150 mg fish oil added, while other doses caused a decrease in semen motility and viability (Table 2).

Another parameter of the study was to determine of the integrity of membrane frozen-thawed. Based on the data on Table 2 in this study, fish oil had no significant effect on membrane integrity.

4. Discussion

AI programs in large livestock including Kalang swamp buffaloes generally use frozen semen. There are various benefits of using frozen semen such as easy transportation, kept in long term, and efficient use of bulls to increase the potential of gene. Success of the semen freezing program can faciliate the improvement of swamp buffaloes population, for sperm can be kept for a long time and can be used as superior genetic stock. Sperm motility is a significant parameter in assessing the quality of spermatozoa, as it will affect the success of fertilization. Therefore, it is required to increase the high motility in post-thawing semen.

In this study, the results revealed that addition of concentration 150 mg fish oil supplementation in extender remarkably enhanced sperm motility before freezing. This significant increment in post-thawed motility at dose 150 mg fish oil supplementation may be related with the collective effect of chemical structure of fish oil. Thus, the presence of concentration in the extender before freezing has provided better protection to the sperm motility or viability against cryopreservation-induced damages.

Sperm qualities including motility can be protected by fish oil during freezing process, because it is related to the content of ingredients from fish oil such as eicosapentaenoic acid, docosahexaenoic acid, saturated fatty acid, and PUFA. Guthrie and Welch[18] explained that the freezing process of semen can cause physical damage and disfunction due to excessive generation of reactive oxygen species. Furthermore, Nair *et al*[19] and Andrabi[8] revealed that pre-dominance of PUFA in sperm plasma membrane instigates the lipid peroxidation process which in turn reduces the semen qualities including sperm viability and motility. While, the part content fish oil of PUFA, eicosapentaneoic acid and docosahexaneoic acid allegedly play a role in sperm resistance to cooling and freezing procedures and may be correlated with the type of long chain PUFA content[20].

While, the previous study[14] conducted on bovine semen revealed a significant progress in post-thawing viability and sperm motility with the addition of 100 mg fish oil in the 100 mL skim milk-eggyolk extender. That is because one of the constituents of fish oil is PUFA with long chains and has been found in the semen of different species including man, bull and ram. These fatty acids could increase the fluidity of the membrane of plasma which is then accountable for improved struggle of the sperm to conditions of cold[21]. The membrane of plasma is the central location of injury prompted by cryopreservation that issues phospholipids into the nearby medium through of cold shock and becomes transiently leaky because of lipid phase transitions[22].

In the study, as for the integrity of membrane of frozen-thawed semen, membrane integrity has not significant effect between control and all treatments after addition concentration of fish oil

Table 1

Effect of fish oil on viability, motility, abnormality and plasma membrane integrity before freezing in Kalang swamp buffalo bull semen.

Treatments	Viability	Motility	Abnormality	Plasma membrane integrity
0 (control)	60.25±0.51	58.25±0.51^{b}	17.50±2.52	61.51±1.96
50 mg fish oil	59.75±2.15	53.75±2.15^{a}	18.52±1.15	63.59±0.95
100 mg fish oil	60.39±0.15	53.57±1.15^{a}	17.25±0.56	62.25±1.28
150 mg fish oil	62.13±1.30	60.13±1.30^{b}	17.05±1.54	61.75±1.46
200 mg fish oil	61.26±2.10	50.26±3.10^{a}	18.75±1.55	62.15±1.70

Different superscripts along the column indicate the significant differences (P < 0.05) among groups (n= 24).

Table 2

Effect of fish oil on viability, motility, abnorality and plasma membrane integrity of post-thawed Kalang swamp buffalo bull semen.

Treatments	Viability	Motility	Abnormality	Plasma membrane integrity
0 (control)	51.20±1.13^{b}	42.25±0.51^{b}	27.50±2.52	59.51±1.96
50 mg fish oil	43.23±1.52^{a}	36.85±2.15^{a}	25.52±1.07	58.98±0.95
100 mg fish oil	44.17±1.15^{a}	36.57±1.15^{a}	26.25±0.36	58.29±1.28
150 mg fish oil	52.17±2.41^{b}	43.13±1.30^{b}	23.05±1.24	57.05±1.46
200 mg fish oil	42.16±1.70^{a}	36.06±3.10^{a}	23.75±1.67	58.16±1.70

Different superscripts along the column indicate the significant differences (P < 0.05) among groups (n=24).

in the extender. These results are confirmed by previous reports by Abavisani *et al*[23] who revealed that the addition of PUFA straightly to extenders was not effective in protection of the sperm membrane.

In conclusion, addition of 150 mg in 100 mL skim milk-egg yolk extender can give a good result to the sperm motility of post-thawed Kalang swamp buffalo semen.

Conflict of interest statement

We declare that we have no conflict of interest.

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