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Effects of Additional Crude Extract of Fig Fruit (*Ficus carica L*) into Tris Egg Yolk Based Extender on Quality of Buck Semen

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Abstract
This research was to investigate the effect of crude extract of fig fruit (CEF2) added into tris egg yolk (TEY) based extender on sperm quality of Boer cross buck. The collected semen was divided into 8 treatments based on the level of CEF2 in TEY based extender (0% CEF2 to 7% CEF2, v/v, respectively). Progressively motile sperm, live sperm, plasma membrane integrity and normal sperm after re-concentration and preserved at 5ºC were assessed visually at 0 and every 24 hours for 7 consecutive days. The optimum concentration of CEF2 in TEY based extender to preserve progressively motile sperm was 6% when stored at 5ºC up to six days, and significantly higher ($P<0.05$) as compared to 0-3% and 7% CF3E. However, progressively motile sperm in 4%-6% CEF2 were non significant ($P<0.05$) each other. At day 6, additional 4-7% CEF2 into TEY based extender were not significantly different ($P<0.05$) from each other on the percentages of life sperms, but they were significantly higher compared to former extenders. There was a tendency that the highest percentages of plasma membranes integrity was in 6% of CEF2 and significantly higher ($P<0.05$) as compared to 0% and 1% of CEF2. Overall means of normal sperm in extender with 3-6% of CF3E were not significantly difference ($P<0.05$) each other except as compared to the former extenders. In contrast, the normal sperms indicated no significant difference ($P<0.05$) among treatments during this study. The concentration of MDA (ng/mL) in treatment extender was reduced when CEF2 was added to extender and the SOD concentration (u/ml) increased. It is concluded that the addition of CEF2 in TEY based extender provides a positive protection performance to the liquid buck semen quality.
Keywords: fig fruit extract, spermatozoa, quality, buck.

1. Introduction

Intra cervical insemination in goat by using fresh semen has not been as popular as in sheep because fresh buck semen cannot be kept very long at 37°C due to the enzymatic component of the bulbo-urethral gland secretion hydrolyses milk triglycerides into free fatty acids, which adversely affects the motility and membrane integrity of buck spermatozoa (Pellicer-Rubio, 1998). When frozen semen was used, fertilization rate was low because there was inadequate numbers of spermatozoa that was able to pass through the cervix (Maxwell and Hewitt, 1986). it has been known that the pregnancy rates from frozen semen could be increased up to 60 - 70% by a trans-cervical method in which semen is directly sprayed to the oviduct or uterus (Leboeuf, 2008), but this is a very expensive and complicated method (Watson, 2000), needs skillful technique, and has a highly risk to the female. Therefore, the use of liquid semen stored at 0 - 5°C for cervical insemination is a better alternative than fresh or frozen goat semen. It allows semen to be stored for a longer period of time compared to fresh semen and results in higher pregnancy rates compared with frozen semen (Paulenz *et al*., 2003; Salamon and Maxwell, 2000). In addition to raising the volume, liquid semen should also maintain its fertility before being sprayed into the female reproductive tract during estrus, so that more females can be inseminated from one ejaculate with the same relative fertility as natural mating. Thus, the extender should be able to reduce excessive metabolic activity in the sperm in order to extend the longevity of spermatozoa, provide nutrients for energy, preventing cold stress, prevent the growth of microorganisms and act as a buffer to prevent pH changes due to the formation of lactic acid that are harmful to spermatozoa (Salamon and Maxwell, 2000).

Antioxidant supplement added into preservation medium could improve the ability of extender to preserve semen quality, because antioxidant molecules could reduce the negative impact of oxidative stress during processing and storing (Foote *et al*., 1993). Fig fruit is an organic source of antioxidant but there is limited research investigating the usage of fig fruit extract as a supplement into extender and its potential effects on goat sperm quality.

Based on the preliminary study, Indonesian local crude extract of fig fruit (*Ficus glumerata* R) indicated positive impact on progressive motility of goat sperm (Zaenuri *et al*., 2013), we hypothesized that the usage of crude extract of Fig Fruit (*Ficus carica L*) or hereinafter referred CEF2 as a supplement into Tris Egg Yolk (TEY)

based extender might help to preserve buck semen quality. Therefore, the present study was designed to evaluate the effects of adding different concentrations of CEF2 into TEY based extender on some *in vitro* quality parameters of Boer cross buck liquid semen.

2. Material and Methods

2.1. Crude Fig Fruit Extraction

Crude extract of fig fruit or CEF2 that is used in this study was the extract of fig fruit that were obtained through the following process. Some ripe fig fruits were cut into slices and then blended. Fig fruit slurry was centrifuged at 3.500 rpm as long as 30 minutes. Supernatant was collected from the glass tube and filtered with 0.20 µm disposable filter unit (Minisart, Sartorius stedim, biotech) and pasteurized at 70ºC for 2 min. then it was kept in refrigerator until use (Zaenuri *et al*., 2013). Nutrient concentrations in fig fruit such as total Tocoferol, α-tocoferol, Vitamin C, β-karotine, Fructose, Glucose, Carbohydrate, Protein, Cu, Mg, Zn and Ca were performed at the Laboratory of Sciences and Food Technology , Bogor Agriculture Institute, Indonesia.

2.2. Experimental extenders

Standard diluents of base extender consisting of 3.634 g tris (hydromethyl) aminomethane (Sigma, USA), 2.17 g citric acid (monohydrate) (Merck, Germany) and 0.50 g fructose (Merck, Germany), 0.06 g sodium penicillin G (Wonder, Japan), 0.1 g streptomycin (Wonder, Japan) were added to 100 ml of distilled water of pH 7.0 was used as a buffer for a standard experimental extender. The 2.5% of buffer experimental extender was dried and replaced with chiken egg yolk at the same volume to be used as based experimental extenders (Evans and Maxwell, 1993). This solution then was called tris citrate egg yolk based extender (TEY). The 0%-7% of the extender from four TEY tubes was dried and replaced with CEF2 at the same volume as dried extender, respectively.

2.3. Semen collection

Ten ejaculates were collected from a healthy and proven fertile Boer cross buck (36 months old and weighing 75 kg) by artificial vagina at 7 day intervals. Ejaculates were immediately sent to Laboratory for initial evaluation (volume, motility, concentration). The quality of each ejaculate that were needed : more than 0.8 ml in volume, more than 70% in motility and 2.5 - 3 × 10^9 spermatozoa/ml in concentration, to be suitable for a further process (Susilawati, 2012).

2.4. Spermatozoa re-concentration and preservation

Sperms were re-concentrated to 100 × 10^6/ml. To minimize osmotic shock, one half of the required TEY for each experimental extender tube was added slowly to the ejaculate, the other half of TEY was added 20 minutes later (Mollineou *et al*., 2011). Each experimental extender tube was separated into 7 aliquots, the same as the number of investigating days. The aliquots were cooled to 5ºC over one hour by placing them into a 200 ml beaker containing 50 ml cooled water (18 – 21ºC), then stored into a 5^{o}C refrigerator until use (Menchaca *et al*., 2010).

2.5. Sperm evaluation

Sperm evaluation was done by observing at least 200 spermatozoa in 10 different observation fields of a phase-contrast microscope (Nikon, Japan) in 400 × magnification. Liquid semen evaluation was performed immediately after re-concentration then every 24 hours for 7 consecutive days. A drop of the prepared sample was placed on a clean sterilized dry glass slide and covered with a cover slip. The percentages of progressively motile sperms were the number of sperm that progressively motile from those of 200 counted sperms (Susilawati, 2012; Menchaca *et al*., 2010).

The percentages of sperms viability and abnormality were observed after stained semen smears that were prepared by mixing diluted 10 µl semen samples with 10 µl nigrosin eosin stains for 30 seconds before preparing smears on microscope slides and then leaving them to be dry. The clear heads and tails were defined as live sperm, while the pink heads and tails were defined as dead sperm. Abnormal sperms were indicated by tailless, abnormal heads and abnormal tail formation with a proximal cytoplasmic droplet and abnormal tail formations with a distal droplet (Evans and Maxwell, 1993). The sperms membrane integrity was evaluated by a hypo-osmotic swelling test (HOST) based on swollen or coiled tails. This was performed by incubating 100 µl of semen sample with 3 ml of a 150 mOsmol/kg hypo-osmotic solution (7.35 g natrium Citrate $_2H_2O$, 13.52 g fructose diluted in 1000 ml of distilled water) at 37ºC for 30 minutes. A drop of the prepared sample was placed on a dry glass slide and covered with a cover slip. Swollen or coiled tails were considered as intact and active sperm membranes, while unswollen head and uncoiled tails were considered as disrupted and inactive sperm membranes (Susilawati, 2012).

2.6. Superoxide dismutase (SOD) and Malondialdehyde (MDA) concentration

Three out of the most optimum concentration of CEF2 to preserve liquid semen quality then analyzed for the concentration of superoxide dismutase (SOD) and Malondialdehyde (MDA). Xanthine oxidase method was performed to determine SOD activity. Samples were detected with a spectrophotometer at 550 nm. Calculated results were presented by U/ml nitrite unit. The content of MDA was measured by thiobarbituric acid method at

absorbance of 532 nm, with results presented by ng/ml protein. Methods and procedures protocol of assay kits were performed according to the manufacturer's instructions.

2.7. Statistical analysis

The statistical significance of the result was evaluated by a two way completely randomized blocks design analysis of variance using CoStat for windows statistical software (version 6.303). Data presented as Mean±SD. Probability P<0.05 considered significantly different.

3. Results

Average viscosity of all extender were normal, average ph were 7.1 for based extender + 0% - 6% CEF2 and 6.9 for TEY + 7% CEF2, respectively. Data on the effect of additional CEF2 to TEY based extender on the percentage of progressively motile and live sperm at day 0 to day 6 post extended and stored at 5°C are presented in Table 1. The percentage of progressively motile sperm at 6 days after preservation, maintained optimally in TEY+6% CEF2 extender and was significantly higher (P<0.05) as compared to the TEY, TEY + 1, 2, 3 and 7% CF3E extenders, difference between the former two extenders was not significant.

Table 1. Percentage progressively motile and live sperm in TEY based extender supplemented by CEF2 at 5°C.

Variables	CEF2 (%)	Preservation days							Mean±SE
		0	1	2	3	4	5	6	
Progressively motile sperm	0	79.1±2.4^{a}	70.4±9.3^{bc}	63.8±8.9^{b}	53.4±10.5^{c}	45.2±12.1^{c}	34.3±9.2^{c}	28.2±8.3^{c}	53.4±8.7^{d}
	1	79.5±1.7^{a}	69.6±6.9^{c}	62.5±8.7^{b}	52.6±9.0^{c}	45.2±8.4^{c}	34.4±6.3^{c}	28.6±5.0^{c}	53.2±6.6^{d}
	2	78.6±2.2^{a}	70.1±7.5^{bc}	62.4±8.0^{b}	55.3±6.4^{bc}	46.6±6.3^{bc}	38.5±11.2^{bc}	30.9±9.8^{bc}	54.7±7.3^{d}
	3	78.9±2.1^{a}	75.0±3.8^{ab}	67.8±5.2^{ab}	60.2±5.5^{ab}	49.1±7.2^{abc}	40.0±5.3^{bc}	33.8±6.3^{bc}	57.8±5.1^{c}
	4	79.8±2.4^{a}	73.4±5.5^{abc}	67.7±6.0^{ab}	61.1±6.6^{ab}	54.2±5.4^{a}	46.9±7.0^{a}	42.3±6.4^{a}	60.8±5.6^{ab}
	5	79.7±1.2^{a}	75.8±4.0^{a}	70.8±5.3^{a}	62.8±3.4^{a}	51.9±5.9^{abc}	47.6±4.9^{a}	43.2±3.6^{a}	61.7±4.0^{ab}
	6	80.1±2.4^{a}	78.2±3.4^{a}	71.2±6.6^{a}	65.9±7.5^{a}	54.0±7.6^{c}	48.6±6.2^{a}	43.9±5.5^{a}	63.1±5.6^{a}
	7	79.4±3.0^{a}	77.3±3.8^{a}	67.9±6.3^{ab}	63.5±6.7^{a}	53.4±6.7^{c}	44.0±4.6^{ab}	35.1±4.1^{b}	60.1±5.0^{bc}
Sperm viability	0	77.1±8.3^{a}	73.6±7.0^{a}	69.1±7.3^{ab}	64.9±7.5^{a}	57.9±7.3^{c}	51.3±7.8^{c}	48.4±7.7^{cd}	62.7±7.6^{d}
	1	78.1±5.3^{a}	73.3±4.6^{a}	68.3±5.8^{b}	65.5±6.4^{a}	59.3±5.5^{bc}	54.5±7.4^{abc}	47.1±10.7^{d}	63.9±6.5^{cd}
	2	79.1±3.1^{a}	75.4±4.8^{a}	70.1±5.7^{ab}	65.9±8.3^{a}	59.6±9.2^{abc}	53.0±12.5^{bc}	45.3±10.9^{d}	64.2±7.8^{cd}
	3	78.3±6.8^{a}	75.1±8.0^{a}	69.5±5.6^{ab}	67.0±6.6^{a}	62.3±7.1^{abc}	55.2±5.3^{abc}	51.1±6.4^{cd}	65.5±6.5^{c}
	4	81.9±3.7^{a}	73.9±8.6^{a}	75.0±5.4^{a}	67.5±8.0^{a}	65.3±4.6^{ab}	59.2±7.6^{ab}	56.1±8.0^{ab}	66.2±6.6^{bc}
	5	79.5±7.1^{a}	75.6±7.5^{a}	71.2±7.7^{ab}	69.3±7.8^{a}	65.3±8.6^{ab}	60.3±10.5^{ab}	58.3±7.5^{a}	68.1±8.1^{ab}
	6	81.3±4.3^{a}	76.7±6.1^{a}	72.9±7.5^{ab}	69.9±8.0^{a}	66.0±7.9^{a}	61.7±9.7^{a}	59.0±5.1^{a}	69.5±6.9^{a}
	7	79.2±9.1^{a}	75.1±8.3^{a}	71.9±8.8^{ab}	68.9±7.7^{a}	66.0±7.5^{a}	60.8±7.4^{ab}	55.5±5.8^{ab}	68.2±7.8^{ab}

The values in the same column with different superscripts differ significantly (P < 0.05)

The effects of additional CEF2 in TEY based extender on live sperm are presented in Table 1. The percentage of live sperm at 6 days after preservation was significantly higher (P<0.05) when 4 - 7% CEF2 added into TEY based extender as compared to control, 1%, 2% and 3% CEF2. However, difference between all four extender (4-7% CEF2) was non significant each other.

Data pertaining to the effect of difference concentration of CEF2 to TEY based extender on intact plasma membrane and normal sperm at 0 to 6 days post extended and stored at 5°C are presented in Table 2. The highest percentage of plasma membrane integrity at day 6 was significantly higher (P<0.05) in TEY based extender added 6% CEF2 compared to control and 1% CEF2. Diffrence between former six different concentration of CEF2 in TEY based extender were non significantly different (P<0.05) each other.

Data on Table 2 shows the effect of additional CEF2 to TEY based extender on the pecentages of normal sperm. The highest percentages of normal sperm at day 6 was significantly higher (P<0.05) in TEY based extender added 6% CEF2 as compared to control and 7% CEF2. However, overall means of normal sperm in TEY based extender added 2 - 6% CEF2 were non significantly different (P<0.05) each other.

Table 2. Percentage of sperm membrane integrity and normal sperm in TEY based extender supplemented by CEF2 at 5ºC.

Variables	CEF2 (%)	Preservation hours 0	1	2	3	4	5	6	Mean±SE
Sperm membrane integrity	0	78.6±5.2^{a}	71.2±5.1^{a}	66.6±4.9^{ab}	61.9±6.5^{b}	55.3±8.6^{a}	49.3±9.6^{b}	43.9±10.4^{c}	60.1±7.2^{de}
	1	78.4±3.9^{a}	72.6±5.9^{a}	66.9±5.3^{ab}	62.1±7.0^{ab}	56.0±9.9^{a}	52.2±9.4^{ab}	44.8±8.1^{bc}	62.1±7.1^{cde}
	2	76.1±7.9^{a}	71.7±7.6^{a}	65.3±5.3^{b}	60.4±7.4^{b}	55.1±10.4^{a}	50.6±11.5^{ab}	46.9±8.5^{abc}	60.4±8.4^{e}
	3	77.4±4.0^{a}	74.3±3.6^{a}	68.1±5.9^{a}	64.7±7.5^{ab}	58.7±8.8^{a}	53.9±10.1^{ab}	47.9±9.4^{abc}	63.6±7.0^{bcd}
	4	78.8±6.2^{a}	73.7±7.5^{a}	69.5±7.0^{ab}	65.3±6.8^{ab}	61.1±8.2^{a}	56.0±8.9^{ab}	51.9±8.5^{abc}	65.2±7.6^{ab}
	5	80.1±4.9	74.4±7.7^{a}	70.3±7.2^{ab}	65.8±6.9^{ab}	60.6±8.8^{a}	55.9±9.7^{ab}	52.1±9.5^{ab}	65.6±7.8^{ab}
	6	80.5±4.1^{a}	75.4±6.9^{a}	71.5±6.5^{a}	68.2±6.3a	61.6±9.7^{a}	58.3±9.6^{ab}	58.8±9.4^{a}	67.1±7.5^{a}
	7	79.2±3.9^{a}	75.0±7.9^{a}	67.4±7.7^{ab}	66.0±7.3^{ab}	59.2±8.9^{a}	54.5±8.5^{b}	50.6±9.0^{abc}	64.5±7.6^{bcd}
Normal sperm	0	94.1±2.5^{ab}	93.6±1.2^{ab}	93.0±1.3^{a}	92.2±1.2^{a}	91.7±1.3^{a}	90.9±2.6^{a}	84.6±2.0^{c}	89.6±2.5^{c}
	1	94.0±3.6^{ab}	91.6±1.4^{b}	90.2±3.5^{b}	89.5±1.4^{b}	89.1±1.8^{bcd}	88.0±3.4^{bc}	87.6±2.4^{ab}	90.0±2.5^{bc}
	2	93.1±4.2^{b}	92.0±1.6^{b}	90.8±1.2^{b}	89.6±2.1^{b}	88.6±2.7^{cd}	87.5±2.4^{c}	85.6±3.1^{bc}	91.2±1.7^{ab}
	3	94.6±2.3^{b}	93.6±1.9^{ab}	93.0±1.1^{a}	92.4±2.0^{a}	90.6±1.3^{abc}	88.4±2.3^{bc}	85.7±1.3^{bc}	92.1±1.7^{a}
	4	95.6±1.5^{a}	94.6±1.7^{a}	93.4±3.8^{a}	92.3±2.9^{a}	91.1±2.4^{ab}	90.3±2.3^{ab}	89.1±2.3^{a}	92.3±2.4^{a}
	5	95.3±1.1^{ab}	94.4±3.7^{a}	93.6±1.0^{a}	92.7±1.2^{a}	92.0±3.8^{a}	90.0±3.1^{ab}	89.0±4.0^{a}	92.4±2.6^{a}
	6	95.4±2.0^{ab}	94.3±2.1^{a}	93.2±2.0^{a}	92.2±3.6^{a}	91.0±2.1^{ab}	90.1±2.2^{ab}	89.4±2.5^{a}	92.2±2.4^{a}
	7	94.8±2.6^{ab}	92.2±3.4^{b}	90.1±1.9^{b}	88.5±4.7^{b}	87.4±3.1^{d}	86.2±3.0^{c}	84.9±4.4^{c}	89.1±3.3^{c}

The values in the same column with different superscripts differ significantly ($P < 0.05$)

This research found the fig fruit nutrients such as Total tocoferol, α- tocoferol, Total fenol, Vitamin C, Cu and Mg (mg/1000g) were 20.40, 0.81, 266.34, 181.50, 0.15 and 223.53, respectively. β-carotene 0.46(µg /100 g), Fructose, Glucose, Carbohydrate, Protein and Ca (mg/100 g) were 18.20, 17.58, 24.27, 1.27 and 44, respectively.

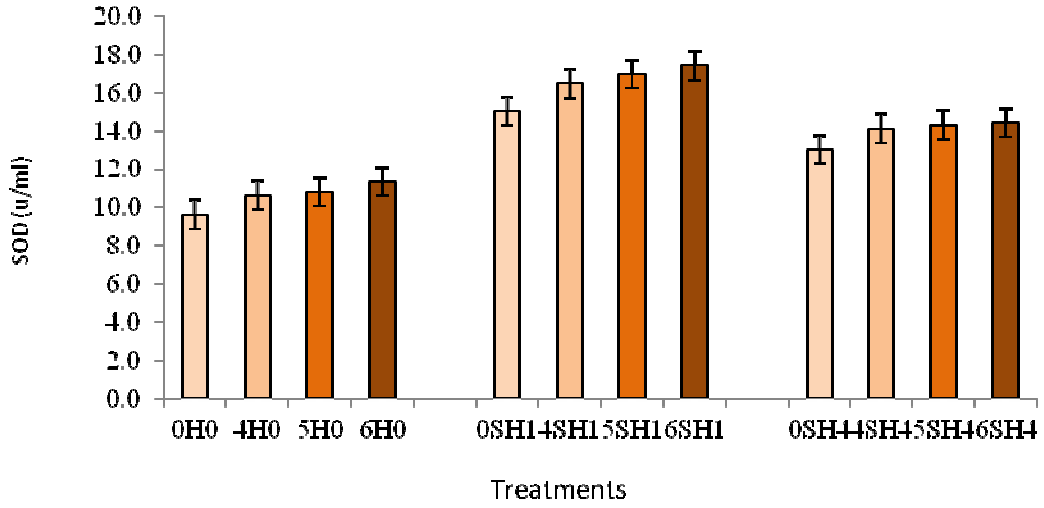

Figure 1. SOD (u/ml) concentration on different concentration of CEF2 pre and post mixing with semen.
Notation 0, 4, 5 and 6 on the left hand side of H = percentages of CEF2 in TEY based extender, S = semen, H0 = 0 hour pre mixing, H1 = 24 hours post mixing and H4 = 96 hours post mixing with semen.

Data on the average extender osmolalities (mOsmol/kg) were 313, 315, 316, 319, 322, 326, 330 and 330 in extender with 0%, 1%, 2%, 3%, 4%, 5%, 6% and 7% CEF2, respectively. The nutrient composition of dried fig fruit are as follows. Total tocoferol, α-tocoferol, Total fenol, Vitamin C, Cu and Mg (mg/1000g) were 20.40, 0.81, 266.34, 181.50, 0.15 and 223.53, respectively. β-carotene 0.46(µg /100 g), Fructose, Glucose, Carbohydrate, Protein and Ca (mg/100 g) were 18.20, 17.58, 24.27, 1.27 and 44, respectively.

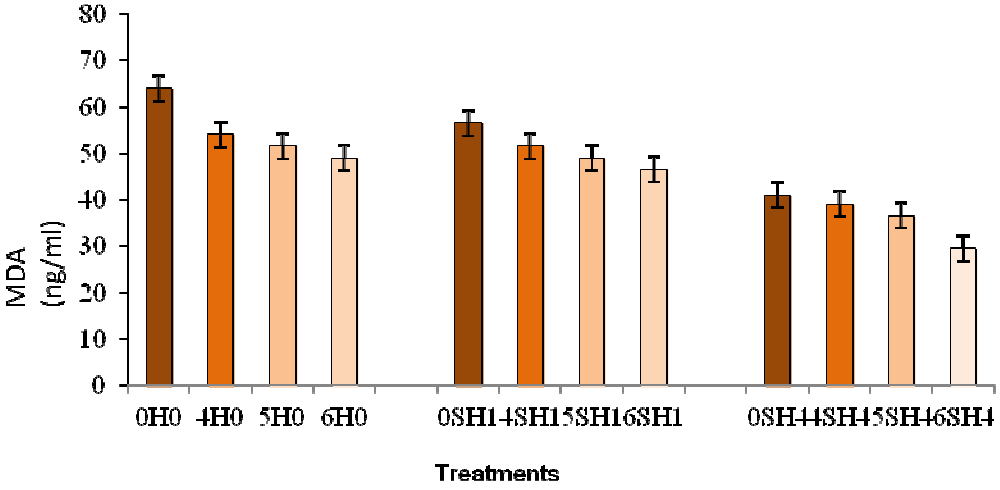

Figure 2. MDA (ng/mL) concentration on different concentration of CEF2 pre and post mixing with semen. Notation 0, 4, 5 and 6 on the left hand side of H = percentages of CEF2 in TEY based extender, S=semen, H0 = 0 hour pre mixing, H1=24 post mixing and H4 = 96 post mixing with semen.

The concentration of Malondialdehyde (MDA) (ng/mL) in all treatment pre-mixed with semen, 1 day and 3 days post mixing with semen, indicated that CEF2 added into TEY based extender decreseased concentration of MDA (Figure 2). Superoxide dismutase (SOD) concentration (u/ml), in contrast, indicated that CEF2 added into TEY based extender increased concentration of SOD (Figure 1).

5. Discussion

In the present study, inclusion of CEF2 did not affect extender osmolalities. The normal extender osmolarities for goat spermatozoa is within 125 mOsm/l (Joshi *et al*., 2006) to 300 mOsm (De Pauw *et al*., 2003). Moreover, additional CEF2 in tris egg yolk citrate based extenders improve the overall means of progressively motile, viability, plasma membrane integrity and normal sperm of buck sperm at 0 to 144 hours after preserved at 5°C. This means that, pre-requisites for long-term preservation of buck semen have been fulfilled. The protective mechanism of CEF2 to preserve sperm quality has yet to be elucidated, but it could be attributed to the synergistic action of all nutrients found on fig fruit.

In this study, the increasing overall mean percentages (Table 1) of progressively motile sperm as a result of the inclusion of CEF2 into TEY base extender might be due to the increasing exogenous antioxidants. This result is in agreement with Zaenuri *et al*. (2013) that, incorporation of CEF2 (*Ficus glumerata Robb*) in TEY based extender increased the sperm motility of liquid Boer cross buck semen. Similarly, the sperm motility of buffalo at 0 and 6 hours after thawing and incubation at 37°C improved due to the additional of vitamin E and C (Andrabi *et al*., 2008). Therefore, not only may the CEF2 supplement in TEY based extender documented in this study increase the concentration of non enzymatic antioxidant in seminal plasma, and provide nutrients for spermatozoa during *in vitro* storage, but also buffer their metabolic by-products. Thus, the protective and preservative effect of the extender might also increase. This hypothesis is supported by previous work (Aurich *et al*., 1997) that, the capacity of endogenous antioxidants may not be sufficient to prevent lipid peroxidation during in vitro storage. As a result, addition of antioxidant supplement into the extender has been suggested to overcome this deficit during dilution. Foote *et al*. (2002) and Aurich *et al*. (1997) suggest that, addition of antioxidant supplement such as α-tocoferol, the main compound in the spermatozoa antioxidant system, into extender could reduce the negative impact of free radicals where the molecules of antioxidant could minimize oxidative stress and then preserve the quality of liquid or post thawing frozen semen.

In this study, the overall mean percentages mean of sperm viability (Table 1), sperm membrane integrity and normal sperm (Table 2) may vary with concentration of CEF2 in TEY based extender. However, when 5 – 6% CEF2 was added into TEY based extender, the percentage of sperm viability, sperm membrane integrity and normal sperm were significantly higher when compared to the control and other extenders. This result supported by Andrabi *et al*. (2008) that additional vitamin E and C significantly increased extender ability to preserved viability and sperm membrane integrity of bull spermatozoa.

In the present study, addition of 5 – 7% of CEF2 into CEY based extender may have been optimum concentration for preserving sperm viability (Table 1), plasma membrane integrity and normal sperm (Table 2) of Boer cross buck sperm. To distinguish effectiveness of CEF2 in extender to provide non enzymatic antioxidants protection system in liquid semen is through Malondialdehyde (MDA) and Superoxide dismutase (SOD) assessment. MDA, the end product of lipid peroxidation is a very reactive compound which provides a

simple and useful tool as a biological biomarker in oxidative stress prediction (DeZwart *et al*., 1998), in which at the increase of MDA concentration leads to the increase of oxidative stress (Stroncek *et al*., 2000). When the concentration of free radical is higher than the concentration of antioxidants then oxidative stress will be occurred followed by increasing concentration of MDA as a result of lipid peroxidation (Short, 2004). SOD, in contrast, is the most important antioxidant system and acts to neutralize the effect of superoxide's and plays a role to protect cells from the negative impact of free radicals in the metabolic system of living organisms and to protect cells from damage (Kelvin *et al*., 2005). In short, the higher SOD concentration the more effective the antioxidant at neutralizing free radicals.

This study confirmed that the addition of CEF2 into TEY based extender was effective in suppressing the formation of free radicals, so that sperm quality could be longer maintained. This study demonstrated CEF2 has a positive role in neutralizing free radicals which was reflected by elevated levels of SOD (Figure 2) and in contrast with levels of MDA (Figure 2) in accordance with the treatment. Moreover, the higher MDA level (Figure 2) in control noteably caused some reduction in progressively motile sperm, viability and plasma membrane integrity. In contrast, the higher percentages of progressive motile sperm, viability and plasma membrane integrity in based extender incorporate with CEF2 being associated with high SOD level (Figure 1). This hypothesis, supported by previous study (Hsieh *et al*., 2003) that, MDA concentrations correlate negatively to the concentration and motility of spermatozoa. Therefore, the activity of SOD and the changes of MDA concentration are not only a reflection of oxygen free radical scavenging, but also as a reflection of the extent of cell damage as the effect of free radicals (Surapaneni and Venkataramana, 2007).

The longevity of sperm quality preserved in TEY based extender enrich with CEF2 was not only due to the high exogenous antioxidants but probably as a respect of the high concentration of fructose in fig fruit, and it may be beneficial to the sperm cell. Hafez (2008) explained that, fructose in goat seminal plasma was significantly lower (250 mg/100 ml) compared to fructose in cattle seminal plasma (460 - 600 mg/100 ml). This was not sufficient to support physiological activity for liquid or frozen buck semen. As a result, goat spermatozoa suffered loss of energy and lifelessness faster than that of cattle spermatozoa. Finally, the higher mean of overall percentage of progressively motile sperm, sperm viability, plasma membrane integrity and normal sperm in TEY based extender with 4%, 5% and 6% rather than in others found in this study could be attributed to the optimum concentration of nutrients that were found in those extenders.

5. Conclusion

Addition of CEF2 into TEY based extender has a positive effect on liquid buck semen quality during storage. However, the exact mechanism of action has yet to be elucidated. Therefore, more comparative studies are necessary to develop a chemically defined extender for buck liquid semen.

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