Effects of Addition of Juice Date Palm to the Extender on the Semen Qualities of Frozen Thawed in Bull Spermatozoa

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Effects of Addition of Juice Date Palm to the Extender on the Semen Qualities of Frozen Thawed in Bull Spermatozoa

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Abstract: The objective of the present study was to investigate the effects of supplementation of juice date palm to the extender on the motility, viability and membrane integrity of frozen thawed bull spermatozoa. Juice date palm was added at the concentration of 0.1%, 0.2%, 0.3% and 0.4% to bovine semen cryoprotective medium of four Bali bull cattle. The cryoprotective extender for the control group was the same as that for the treatment groups except that it was not supplemented with juice date palm. The results indicated that percentage of the sperm viability, motility, abnormality and membrane integrity on the fresh sperm were no significantly different ($P > 0.05$) among control and treatment groups. Furthermore, the percentage of the motility, abnormality and membrane integrity during freezing was no significantly different ($P > 0.05$) among control and treatment groups. Whereas, the viability before freezing was significantly higher ($P < 0.05$) between control and treatment groups. The percentage of the viability, abnormality and membrane integrity of frozen thawed was no significantly different ($P > 0.05$) among control and treatment groups. However, the motility of frozen thawed was significantly lower ($P < 0.05$) between control and treatment groups. The percentage of sperm viability before freezing was higher compared to that of without substitution on the juice date palm. Furthermore, the percentage of sperm motility frozen thawed were lower compared to that of without substitution on the juice date palm.

Key words: Juice date palm · Spermatozoa · Viability · Motility and membrane integrity

INTRODUCTION

Date palm is one of the oldest fruit crops grown in the Arabian Peninsula, North Africa and the Middle East. Fruits of date palm (*Phoenix dactylifera L*) is rich in mineral salts and vitamins [1]. Date palm contain small amounts of vitamins C, B1 thiamine, B2 riboflavin and nicotinic acid [2]. Al Farsi *et al.* [3] reported that dates palm also have strong antioxidant. Date palm fruits consist of 3 main parts: date flesh, date pit and skin. The glucose, fructose and sucrose are the main sugars of date flesh. The palm date fruit has a high content of sucrose at early stages of maturing, but during the maturation process it is converted to glucose and fructose. Proteins perform in date fruits is 1-3% of dry matter, while, fat content of date palm was 0.52–3.25% [4].

Several studies were revealed on the effects of date palm parts, such as date extracts, gemmule and date pits on the reproductive organs [5, 6].Whereas, Pollen of date palm has been used for a herbal medicine for improving male and female fertility[7]. Date palm gemmule also was used in traditional medicine to improvment the quality of semen and treat infertility [8]. Existing sugar palm juice is an important ingredient for spermatozoa quality including viability and motility. Thus, the objective of this study was to investigate the effects of addition juice date palm to the extender on the viability, motility, abnormality and membrane integrity of frozen thawed bull spermatozoa.

MATERIALS AND METHODS

Semen Collection: This study was conducted at the artificial insemination center Banjarbaru south Kalimantan

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Indonesia. A total of four Bali bull cattle were used to the study. The semen was collected from the Bali cattle bulls as long as two month with the aid of an artificial vagina.Semen was kept in a water bath (37°C) immediately after collection and volume, pH, consistency, color and concentration of the spermatozoa were assessed. The extender was composed of skim milk, glucose, egg yolk and glycerol according to Bustamante *et al*. [6] and was supplemented with different concentrations of juice date palm (0.1%, 0.2%, 0.3% and 0.4%). The cryoprotective extender for the control group was the same as that for the treatment groups except that it was not supplemented with juice date palm. Juice date palm supplement was made by Amal Mulia Sejahtera (AMS) Company, Bogor, Indonesia to obtain 120 × 10^6 sperm/mL.

Semen Cryopreservation: Semen was loaded in 0.25 mL straws (Biovet, France) and maintained at 4 °C for 2 hours before freezing. Then they were frozen at 4 cm above liquid nitrogen to achieve approximately -120°C for 10 min before being I mmersed into liquid nitrogen tank [9] and stored at least 2 weeks before thawing were analyzed.

Sperm Viability: The viability of the spermatozoa was used to assess as described by Suherni *et al*. [10]. One drop of fresh and thawed semen was located on a glass slide and mixed with one drop of eosin-nigrosin solution (0.2 g of eosin and 2 g of nigrosin were dissolved in a buffered saline solution, mixed for 2 hours at room temperature and filtered to obtain the staining media). The mixture was smeared on the glass slide and allowed to air dry. One hundred spermatozoa were evaluated in five different fields in each smear under a light microscope. Eosin penetrates non-viable cells, which appear red and nigrosin offers a dark background for facilitating the detection of viable, non-stained cells.

Assessment of Motility: Calculation of Motility of the spermatozoa was examined with mixing the semen gently and placing a 10-µL drop of diluted semen on a warm slide covered with a glass cover slip from five selected representative fields. Samples were selected randomly from 10 fields, for a total of 200 cells. Individual sperm were noted as being viable or dead.

Assessment of Sperm Membrane Integrity: The membrane integrity was determined using the hypo-osmotics welling test (HOST). A total of 100 µl of fresh and after thawing semen was mixed with 1 mL of hypotonic solution (Osmotic pressure 100 mOsm/kg) containing 13.51 g of fructose and 7.35 g of sodium citrate in 1000 mL of distilled water. The mixture was incubated at 37 °C for 60 min. Following incubation, 15 µl of the sample was placed on a slide, covered with a cover slip and observed under a differential interference microscope (Olympus CK2, ULWCD 0.30) at a magnification of 400x. At least 200 spermatozoa were observed and the results were recorded as percentages. The membrane integrity was classified into two groups: the normal spermatozoa that displayed coiled tails and abnormal spermatozoa without coiled tails [11].

Abnormalities of Spermatozoa: The abnormalities of sperm were evaluated based on classified morphological abnormalities into the categories such as loose spermatozoa head, abnormal spermatozoa head and tail formation, presence of proximal cytoplasmic droplet, or distal cytoplasmic droplet adopted from Hafez and Hafez [12].

Statistical Analysis: Percentage of mean values (± SEM) for several parameters of semen quality including fresh and frozen thawed were calculated using SPSS. The statistical significances of the effects of viability, motility and membrane integrity for fresh and frozen thawed were determined by ANOVA (S-PLUS Statistical Program, Insightful Corporation Seattle, WA, USA).

RESULTS

The effect of addition juice date palm to the extender on the percentage of the viability, motility, abnormality and membrane integrity of fresh semen Bali bull spermatozoa was showed in Table 1. Compared to the control the percentage of the sperm viability, motility, abnormality and membrane integrity on the fresh sperm was no significantly different ($P > 0.05$) among control and treatment groups. The percentage of the viability, motility, abnormality and membrane integrity before freezing was showed in Table 2. The percentage of the motility, abnormality and membrane integrity during freezing was no significantly different ($P > 0.05$) among control and treatment groups. Whereas the viability during freezing was significantly higher ($P < 0.05$) between control and treatment groups. The percentage of the viability, motility, abnormality and membrane integrity of frozen thawed Bali bull spermatozoa was showed on Table 3. The percentage of the viability, abnormality and membrane integrity of

Table 1: The sperm quality on fresh including motility, membrane integrity, abnormality and viability bali bulls spermatozoa

		Concentration of juice date palm (%)			
Item	Control	0.1	0.2	0.3	0.4
Motility (%)	73±2.43	75±1.05	74±4.12	72±2.57	72±1.90
Membrane integrity (%)	73±1.67	71±5.23	72±0.13	69±2.89	70±2.54
Abnormality (%)	15±0.51	15±9.51	16±0.01	15±9.85	16±0.11
viability (%)	81±3.08	82±4.12	82±1.56	80±5.23	79±2.13

Table 2: The sperm quality before freezing including motility, membrane integrity, abnormality and viability bali bulls spermatozoa

		Concentration of juice date palm (%)			
Item	Control	0.1	0.2	0.3	0.4
Motility (%)	58.00±3.56	59.25±2.50	54.50± 4.43	53.25±5.19	50.00±5.66
Membrane integrity (%)	72.25±5.56	68.50±3.11	66.00±5.94	61.75±4.75	58.00±4.40
Abnormality (%)	17.13±1.56	17.11±8.11	17,02±2.00	18.02±6.19	18.31±6.34
Viability (%)	79.5±4.23^{a}	81.75±6.45^{ab}	86.75±3.59^{abc}	84.75±9.18b^{c}	81.50±1.03^{c}

^{a,b,c}Values in the same colum with different superscripts indicate significant difference (P<0.05) (n=24)

Table 3: The sperm quality of frozen thawed including motility, membrane integrity, abnormality and viability bali bulls spermatozoa

		Concentration of juice date palm (%)			
Item	Control	0.1	0.2	0.3	0.4
Motility (%)	43.50±1.00^{a}	41.25±1.26^{ab}	39.75±1.26^{b}	36.00±1.41^{c}	31.00±2.94^{d}
Membrane integrity (%)	62.50±6.66	61.25±1.14	54.75±1.01	53.75±1.56	50.00±6.88
Abnormality (%)	18.01±2.61	19.32±5.56	18.01±15.46	19.90±6.86	19.89±4.16
Viability (%)	61.75±4.06	68.50±1.02	67.50±1.96	61.75±1.78	65.25±3.25

^{a,b,c,d}Values in the same colum with different superscripts indicate significant difference (P<0.05) (n=24).

frozen thawed was no significantly different (P>0.05) among control and treatment groups. However, the motility of frozen thawed was significantly lower (P < 0.05) between control and treatment groups.

DISCUSSION

The assessment of the viability, motility, abnormality and membrane integrity is one of the most often used parameters for semen evaluation in the bull. The results of this study indicate that average percentage of the viability, motility, abnormality and membrane integrity in fresh semen was similar compared with control.

Viability of sperm is essential to assess the structural and functional activity of the spermatozoa membrane for the viability and ability of fertilization of spermatozoa [12]. Moreover, the percentage of sperm motility, abnormality and membrane integrity before freezing was no significantly different compared with control. Whereas, the percentage of sperm viability on the treatment was higher compared with control. This result strengthened the findings reported by Assirey[13]in which the juice date palam is an excellent material for producing refined sugar.

Other studies showed that sugars could interact directly with membrane lipids and proteins, altering their phase transition behavior and hydration state [14]. Sugars also protect against the injury occurring to sperm cells during freezing-thawing, although this protection depends on many factors, such as storage temperature, molecular weight of sugar and the type of buffer used [15]. Furthermore, Purdy [16]revealed that sugar in the extender supplied energy to maintain semen quality. The quality of spermatozoa can be maintained through the use of different extenders, which interact with spermatozoa to provide protection during the cooling, freezing and thawing processes [17].

Evaluation of sperm quality including motility, abnormality and membrane integrity is essential factors because these parameters are important for cell survival and fertilization potential in cattle [18-20]. The motility is one of the most essential factors in evaluating bull and other sperm of mammalian because it contributes information about the spermatozoa cell's energy sources [21]. In our study, the percentage of sperm viability, abnormality and membrane integrity frozen thawed were no significantly different compared with control. Whereas, the percentage of sperm motility on the treatment was

lower than control. These findings confirmed several studies reported by Haugana *et al*. [22] and Hong *et al*. [23] who stated that the cryopreservation is a major cause of damage to the sperm thawed. These was probably due to the induction of reactive oxygen species (ROS) produced from cryopreservation can also induce of damage sperm thawed [24, 25]. On the other hand, mitochondria are the source of sperm energy and damage to their structure during the cryopreservation process is associated with reduced post-thaw including sperm viability and motility [26].

CONCLUSSION

The percentage of sperm viability before freezing was higher compared to that of without substitution on the juice date palm. Furthermore, the percentage of sperm motility in frozen thawed was lower compared to that of without substitution on the juice date palm.

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