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Research Article
Effects of Honey Supplementation into the Extender on the Motility, Abnormality and Viability of Frozen Thawed Of Bali Bull Spermatozoa

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Abstract

Background and Objective: Honey is comprise high amount of variety of simple sugars which might serve nutrition to sperm cells. The objective of this study was to evaluate the effects of honey addition into the extender on the quality of frozen thawed in Bali bull spermatozoa. **Materials and Methods:** A total of four Bali cattle bulls were used in this study. Honey solution was added at the concentration of 0.1, 0.2, 0.3 and 0.4% to bovine semen cryoprotective medium. The cryoprotective extender (skim milk-egg yolk) for the control group was the same as that for the treatment groups except that it was not supplemented with honey solution. Sperm parameters were assessed including motility, abnormality and viability. The data were statistically analyzed pre and post-thawing. **Results:** The results indicated that percentage of the sperm motility before freezing was significantly lower ($p>0.05$) among control and treatment groups. Furthermore, the percentage of the abnormality and viability were no significantly different ($p>0.05$) among control and treatment groups. The sperm abnormality frozen thawed was significantly higher ($p<0.05$) between control and treatment groups. Whereas, the percentage of the motility and viability of frozen thawed was no significantly different ($p>0.05$) among control and treatment groups. **Conclusion:** It is concluded that honey supplementation into the extender was significantly effect on the sperm motility before freezing and sperm abnormality on the frozen thawed.

Key words: Bali bull, semen cryopreservation, honey, viability, motility and abnormality

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of original breed cattle in Indonesia is Bali cattle. The Bali cattle (*Bos sondaicus*), domesticated from *Bos banteng* in Java, have been reported to have higher reproductive performance than other indigenous Indonesian cattle[1]. To raise the genetic improvement and population of local cattle breeds artificial insemination (AI) has become a regular procedure to improve the useful reproductive life of livestock[2]. One of the factor to success of AI is semen quality including a extender. Semen extender is added in order to preserve metabolic of spermatozoa demands, control pH changes in the extracellular environment of the spermatozoa, reduce cryogenic damage and also control bacterial contamination.

Purdy[3] repoted that the greatest components of semen extenders used for cryopreservation include nutrients, egg-yolk, buffer, cryoprotectant and antibiotics, each providing a multifarious and vital role in protecting the spermatozoa. Technique of cryopreservation allows spermatozoa to be stored indefinitely under in liquid nitrogen. However, cryopreservation is known to cause damaging effects to spermatozoa partly through the freezing and thawing process. Bearden *et al.*[4], reported that added nutrients in semen extender are simple sugars such as glucose and fructose. Insufficient nutrients in the semen extender will reduce the metabolic activity of the spermatozoa and lead to greater number of dead spermatozoa[5].

Honey is known to contain high amount of a variety of simple sugars[6] which might serve both as a source of nutrition and non-penetrating cryoprotectant to sperm cells during cryopreservation. Bogdanov *et al.*[7] and Fakhrildin *et al.*[8], revealed that honey contains tiny amounts of numerous compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase and pinocembrin. Furthermore, Erejuwa *et al.*[9] defined honey as a novel antioxidant considering its effect on the health of the various organs of the body from damage due to oxidative stress or reactive oxygen species (ROS). On the other hand, Saxena *et al.*[10], stated that honey is mainly composed of a complex mixture of carbohydrates, proteins, enzymes such as invertase, glucose oxidase, catalase, phosphatases, amino acids and organic acids.

Exiting sugar solution in honey is important ingredient for support spermatozoa quality. Thus, the objective of this study was to investigate the effects of supplementation of different concentration of honey into the skim milk-egg yolk extender on the motility, abnormality and viability of frozen thawed in Bali bull spermatozoa.

MATERIALS AND METHODS

Semen collection: This study was conducted at the artificial insemination center in Banjarbaru South Kalimantan Indonesia during 3 months (January-March 2016). A total of four Bali bull cattle were used to the study. The semen was collected from the Bali cattle bulls as long as 2 month with the aid of an artificial vagina. Immediately, after semen collection was kept in a water bath (37°C) and semen parameters were assessed base on macroscopic and microscopic characteristics. Macroscopic evaluations included volume, pH and color. Microscopic evaluations included spermatozoa motility, abnormality and viability. The extender for this study was composed as follows, skim milk, glucose, egg yolk and glycerol according to Filho *et al.*[11] and was supplemented with different concentrations of honey (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 honey solution.

Semen cryopreservation: Cryopreservation of bulls semen were loaded in 0.25 mL straws (Biovet, France) and maintained at 4°C for 2 h before freezing. Then they were frozen at 4 cm above liquid nitrogen to achieve approximately -120°C for 10 min before being immersed into liquid nitrogen thank 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.*[12]. Fresh and after thawing semen, one drop of semen was located on a glass slide and this sample was 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 h 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 at least 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. Spermatozoa that stained pink-purple due to absorption of eosin-negrosin were considered dead while those that didn't take up the stain (remained white) were considered live.

Assessment of sperm motility: The sperm motility was evaluated by placing a drop of semen on a clean slide and observing under the microscope. Motility was expressed as the percent of motile spermatozoa with rapid forward

movement[13]. Calculation of sperm motility 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 (18×18 mm) from five selected representative fields. Samples were selected randomly from 10 fields, for a total of 200 cells.

Abnormalities of spermatozoa: The eosin-nigrosin stain technique slides were used to determine sperm abnormality. 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[14].

Statistical analysis: The percentage values for fresh and post-thawed semen quality parameters were expressed as Mean±SEM. A one-way analysis of variance (ANOVA) with Tukey's multiple comparison *post-hoc* test was used to determine differences among extenders using the SPSS Statistical Software version 20.0. Differences with a p-value of 5% were considered to be statistically significant.

RESULTS

Based on the evaluation of fresh ejaculation, the mean of semen volume was 6.06±12.41 mL, had a pH of (6.53±0.15) and the sperm concentration was 940.83×10^6 sperm mL^{-1}. The percentage of spermatozoa motility, abnormality and sperm viability are shown in Table 1.

The percentages of spermatozoa motility, abnormality and viability of Bali bull spermatozoa before freezing is shown in Table 2. Before freezing, Sperm motility was significantly lower ($p<0.05$) between control and all treatments. Furthermore, the sperm abnormality and viability were no significantly increased ($p>0.05$) between control and all treatments in Bali bull spermatozoa.

The percentages of motility, abnormality and viability of Bali bull spermatozoa frozen thawed are shown in Table 3. The result of the study shown that sperm abnormality post thawed was significantly increased ($p<0.05$) between control and all treatments. Furthermore, the sperm motility and viability was not significantly increased ($p>0.05$) in frozen thawed in bali bull spermatozoa.

DISCUSSION

One of the parameters of this study is compare between fresh semen and post-thawed percentage of motility, abnormality and viability. The result indicated that sperm motility, abnormality and viability were decreased. These findings confirmed several studies reported by Haugan *et al.*[15] and Hu *et al.*[16], who reported that the cryopreservation is a major cause of damage to the sperm thawed. This was probably due to the induction of reactive oxygen species (ROS) formation by the spermatozoa. These findings confirmed several studies reported by Sharma *et al.*[17] and Alvarez *et al.*[18], who reported that the effects of cryopreservation and frozen thawed attributed to the generation of ROS, which can irreversibly damage the spermatozoa.

Table 1: Characteristics of qualities of fresh semen ejaculation of Bali bull spermatozoa

Characteristic of semen (%)	Mean±SEM
Sperm motility	71.83±3.21
Sperm abnormality	6.35±58.16
Sperm viability	81.59±32.31

Table 2: Quality of spermatozoa before freezing semen including motility, abnormality and viability of spermatozoa

		Concentration of honey (%)			
Item (%)	Control	0.1	0.2	0.3	0.4
Motility	62.61±21.42^{c}	59.36±34.51^{c}	54.31±15.01^{b}	53.06±46.08^{b}	45.56±13.18^{a}
Abnormality	25.12±34.02	26.73±27.26	33.52±19.09	34.67±27.91	36.58±34.91
Viability	72.12±42.19	69.74±18.07	67.39±20.19	64.56±31.08	62.46±10.71

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

Table 3: Quality of frozen thawed including motility, abnormality and viability of Bali bull spermatozoa

		Concentration of honey (%)			
Item (%)	Control	0.1	0.2	0.3	0.4
Motility	45.09±51.01	47.51±27.11	46.18±21.42	44.97±52.07	42.31±15.71
Abnormality	25.55±23.06^{a}	35.07±50.49^{ab}	36.18±09.21^{ab}	39.03±48.05^{bc}	50.16±17.01^{c}
Viability	58.34±34.81	55.45±06.15	55.56±43.01	52.34±41.19	53.02±53.71

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

Sperm motility is defined as percentage of moving cells in any direction at any speed[13]. The spermatozoa which are moving rapidly in straight and forward direction exhibit progressive motility.In this study, percentage of the motility before freezing after honey supplemented into the extender was significantly lower compared the control. Decreasing percentage of sperm motility before freezing on the treatment 0.2 and 0.4% might be due the effect on the ratio of honey-egg yolk in extender and different base ingredients. This result strengthened the finding reported by Yimer *et al.*[19]. Naturally, honey is a highly concentration product and has the potential hyperosmotic extracellular environment around sperm cells. On the other hand, Arifiantini *et al.*[20] revealed that diluent on different base components will affect the sperm to survive *in vitro* fertilization.

Another objective of this study was to evaluate the abnormality frozen thawed. Based on the data in Table 3 in this study, the abnormality was increase gradually during higher level addition of honey concentration. The mechanism of sperm abnormality increase after thawed on this study is not fully understood. These are probably due to deleterious effect of cryopreservation process, including cooling, freezing and thawing[21].

Motility of spermatozoa frozen thawed is an important factor because spermatozoa must travel from the vagina and uterus if semen is deposited by natural mating and artificial insemination, respectively. In this study, the percentage of sperm motility frozen thawed was shown increase (47.51±27.11) on T1 and (46.18±21.42) on T2 compared the control (45.09±51.01). This result strengthened the findings reported by El-Sheshtawy *et al.*[22] and Olayemi *et al.*[23] addition of honey solution to semen extender improved sperm motilty in frozen thawed. Honey is known to consist of primarily sugars such as monosaccharides, disaccharides, oligosaccharides and polysaccharides[6,7] that can act as a source of energy to support spermatozoa survival and motility during cryopreservation and after thawed. This result reinforced the findings reported by Gadea[5], Bearden *et al.*[4] and Purdy[3] sugar is one of the essential components of most semen extenders. Furthermore, Olayemi *et al.*[23] revealed that addition of small proportion of honey in egg yolk extender gave the highest percentage of sperm motility. The study discover the addition honey in the skim milk-egg yolk extender on dose 0.1-0.2% improve sperm motility post-thawed that can be beneficial for sperm quality frozen thawed. As well as, honey can be used cryoprotectants to spermatozoa cells during cryopreservation, because contains small amounts of numerous compounds thought to function as antioxidants, including chrysin, pinobanksin, catalase, vitamin C and pinocembrin.

CONCLUSION

Based on the results of these experiments, it may be concluded that sperm motility before freezing was lower compared between control and treatment of honey supplementation. Furthermore, the percentage of sperm abnormality in frozen thawed was higher between control and treatment of honey supplementation.

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REFERENCES

1. Malik, A., A. Gunawan, A. Jaelani, R. Budirahman and S. Erlina, 2013. Comparison of estrus synchronization with application of prostaglandin $f_2\alpha$ intrauterine and intramuscular in bali and crossbred-ongole cattle. Pak. Vet. J., 33: 446-449.
2. Gonzalez, R., M.A. Sanchez, P. Garcia-Palencia, B. Sanchez, R.A. Garcia-Fernandez, A. Gonzalez-Bulnes and J.M. Flores, 2012. Differences in uterine immunoexpression of PR, ERα and OTR when comparing prostaglandin- to progestagen-based protocols for ovine estrus synchronization. Anim. Rep. Sci., 133: 93-100.
3. Purdy, P.H., 2006. A review on goat sperm cryopreservation. Small Rumin. Res., 63: 215-225.
4. Bearden, J.H., J.W. Fuquay and S.T. Willard, 2004. Applied Animal Reproduction. 6th Edn., Pearson Education, Inc., Upper Saddle River, New Jersey, pp: 20-24.
5. Gadea, J., 2003. Review: Semen extenders used in the artificial insemination of swine. Spanish J. Agric. Res., 1: 17-27.
6. Fuller, B.J., 2004. Cryoprotectants: The essential antifreezes to protect life in the frozen state. Cryo Lett., 25: 375-388.
7. Bogdanov, S., T. Jurendic, R. Sieber and P. Gallmann, 2008. Honey for nutrition and health: A review. J. Am. Coll. Nutr., 27: 677-689.
8. Fakhrildin, M.B.M.R. and R.AR. Alsaadi, 2014. Honey supplementation to semen-freezing medium improveshuman sperm parameters post-thawing. J. Family Rep. Health., 8: 27-31.
9. Erejuwa, O.O., S.A. Sulaiman and M.S. Ab Wahab, 2012. Honey: A novel antioxidant. Molecules, 17: 4400-4423.
10. Saxena, S., S. Gautam and A. Sharma, 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem., 118: 391-397.

11. Filho, I.C.B., C.D. Pederzolli, A.M. Sgaravatti, R.M. Gregory, C.S. Dutra Filho, M.I.M. Jobim and R.C. Mattos, 2009. Skim milk-egg yolk based semen extender compensates for non-enzymatic antioxidant activity loss during equine semen cryopreservation. Anim. Rep., 6: 392-399.
12. Suherni, S., I.T. Norma and A. Malik, 2015. The effects of insulin-like growth factor I (IGF-I) complex from seminal plasma on capacitation, membrane integrity and DNA fragmentation in goat spermatozoa. Asian Pac. J. Rep., 4: 208-211.
13. Muzaffar, H., T. Khaliq, J.A. Khan, Zia-ur-Rahman and A. Mahmood *et al*., 2016. Effect of protein, probiotics and vitamins supplementation on semen quality and immunohistochemistry of pituitary gland in molted male layer breeders. Pak. Vet. J., 36: 149-152.
14. Hafez, B. and E.S.E. Hafez, 2000. Reproductive Behavior. In: Reproduction in Farm Animals, 7th Edn., Hafez, E.S.E. and B. Hafez (Eds.). Baltimore, MD: Lippincott Williams and Wilkins, pp: 293-306.
15. Haugan, T., Y.T. Grohn, E. Kommisrud, E. Ropstad and O. Reksen, 2007. Effects of sperm concentration at semen collection and storage period of frozen semen on dairy cow conception. Anim. Reprod. Sci., 97: 1-11.
16. Hu, J.H., Q.W. Li, Y.L. Chen, Z.L. Jiang, Y.H. Jia, L.Q. Wang and B.B. Ou, 2009. Effects of addition of vitamin B_{12} to the extender on post-thaw motility, acrosome morphology and plasma membrane integrity in bull semen. Turk. J. Vet. Anim. Sci., 33: 379-384.
17. Sharma, R.K., S. Vemulapalli, S. Kohn and A. Agarwal, 1997. Effect of centrifuge speed, refrigeration medium and sperm washing medium on cryopreserved sperm quality after thawing. Arch. Androl., 39: 33-38.
18. Alvarez, J.G., J.L. Lasso, L. Blasco, R.C. Nunez, S. Heyner, P.P. Caballero and B.T. Storey, 1993. Centrifugation of human spermatozoa induces sublethal damage; separation of human spermatozoa from seminal plasma by a dextran swim-up procedure without centrifugation extends their motile lifetime. Hum. Reprod., 8: 1087-1092.
19. Yimer, N., N. Muhammad, K. Sarsaifi, Y. Rosnina, H. Wahid, A. Khumran and A. Kaka, 2015. Effect of honey supplementation into Tris Extender on Cryopreservation of Bull Spermatozoa. Malaysian J. Anim. Sci., 18: 47-54.
20. Arifiantini, R.I., T.L. Yusuf and N. Graha, 2005. [Longivitas and recovery rate of frozen thawed semen holstein Friesian cattle using different diluent]. Bull. Peternakan, 29: 2-5.
21. Malik, A., M. Laily and M.I. Zakir, 2015. Effects of long term storage of semen in liquid nitrogen on the viability, motility and abnormality of frozen thawed Frisian Holstein bull spermatozoa. Asian Pac. J. Reprod., 4: 22-25.
22. El-Sheshtawy, R.I., W.S. El-Nattat, H.A. Sabra and A.H. Ali, 2014. Effect of honey solution on semen preservability of local breeds of cattle bulls. World Applied Sci. J., 32: 2076-2078.
23. Olayemi, F.O., D.A. Adeniji and M.O. Oyeyemi, 2011. Evaluation of sperm motility and viability in honey-included egg yolk based extender. Global Vet., 7: 19-21.