บทความวิชาการ

สัตวแพทย์มหานครสาร

JOURNAL OF MAHANAKORN VETERINARY MEDICINE

Available online: www.tci-thaijo.org/index.php/jmvm/

การเสริมน้ำผึ้งความเข้มข้นที่เหมาะสมในสารละลายถั่วเหลืองเป็นแหล่งพลังงานในการเคลื่อนที่ของตัวอสุจิโคชนที่เก็บรักษาด้วยการแช่เย็น

วิภาวี แสงสร้อย[1,#] และโฆษิต อารีกิจ[1]

[1]คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเทคโนโลยีราชมงคลศรีวิชัย อำเภอทุ่งใหญ่ จังหวัดนครศรีธรรมราช *80240*

บทคัดย่อ: น้ำผึ้งเป็นผลิตภัณฑ์จากธรรมชาติซึ่งอุดมไปด้วยสารอาหารที่หลากหลาย และให้พลังงานแก่เซลล์ของร่างกาย การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมน้ำผึ้งที่ความเข้มข้นแตกต่างกันในสารละลายน้ำเชื้อที่มีส่วนผสมของเลซิตินจากถั่วเหลือง ต่อการเก็บรักษาคุณภาพน้ำเชื้อโคชนด้วยวิธีการแช่เย็นและแช่แข็ง โดยใช้ตัวอย่างน้ำเชื้อโคชนที่ทำการรีดเก็บด้วยวิธีกระตุ้นด้วยไฟฟ้า แล้วนำไปเจือจางด้วยสารละลายน้ำเชื้อที่มีส่วนผสมของเลซิตินจากถั่วเหลือง โดยทำการเสริมน้ำผึ้งที่ความเข้มข้น 0, 1, 2, 4 และ 8 เปอร์เซ็นต์ สำหรับการทดลองทำน้ำเชื้อแช่เย็นที่อุณหภูมิ 5 องศาเซลเซียส และทำการเสริมน้ำผึ้งที่ความเข้มข้น 0, 1 และ 2 เปอร์เซ็นต์ สำหรับการทำน้ำเชื้อแช่แข็งที่อุณหภูมิ -196 องศาเซลเซียส จากนั้นทำการตรวจคุณภาพน้ำเชื้อ ได้แก่ เปอร์เซ็นต์การเคลื่อนที่โดยรวม การเคลื่อนที่ไปข้างหน้า และการมีชีวิตของตัวอสุจิ ในวันที่ 1 3 และ 5 หลังจากการแช่เย็น และในวันที่ 7 ของการแช่แข็งน้ำเชื้อ โดยใช้สารละลายน้ำเชื้อที่ไม่ได้ผสมน้ำผึ้งเป็นกลุ่มควบคุม ผลการศึกษาพบว่าน้ำเชื้อแช่เย็นกลุ่มที่เสริมน้ำผึ้งเข้มข้น 2 เปอร์เซ็นต์ มีค่าการเคลื่อนที่โดยรวม และการเคลื่อนที่ไปข้างหน้าของตัวอสุจิสูงที่สุดเป็นระยะเวลานานถึง 3 วันของการแช่เย็น เมื่อเทียบกับกลุ่มที่เสริมน้ำผึ้ง 8 เปอร์เซ็นต์และกลุ่มควบคุมอย่างมีนัยสำคัญ อย่างไรก็ตาม ไม่พบการเพิ่มขึ้นของคุณภาพน้ำเชื้อในน้ำเชื้อแช่แข็งที่เสริมน้ำผึ้ง ดังนั้น การศึกษานี้สรุปได้ว่าการเสริมน้ำผึ้งที่ความเข้มข้น 2 เปอร์เซ็นต์ ในสารละลายน้ำเชื้อชนิดเลซิตินจากถั่วเหลือง สามารถเป็นแหล่งพลังงานสำหรับการเคลื่อนที่ของตัวอสุจิ ในระหว่างการเก็บรักษาน้ำเชื้อโคชนด้วยวิธีการแช่เย็น

คำสำคัญ: น้ำผึ้ง สารละลายเลซิตินจากถั่วเหลือง น้ำเชื้อแช่เย็น น้ำเชื้อแช่แข็ง โคชน

[#]ผู้รับผิดชอบบทความ

สัตวแพทย์มหานครสาร. 2565. 17(2): 255-263.

E-mail address: wipawee.s@rmutsv.ac.th

Appropriated Honey Concentration Supplementation in Soy Lecithin Extender as an Additive Source of Energy to Improve Motility of Cool-Stored Fighting Bull Spermatozoa

Wipawee Saengsoi[1,#] and Kosit Areekit[1]

[1]Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya, Thungyai, Nakhon Si Thammarat 80240, Thailand

Abstract: Honey is a natural product enriched in multiple nutrients and provides energy to body cells. The objective of this study was to investigate the effects of honey supplementation in soy lecithin-based extender on both cooled and frozen semen preservation of fighting bulls. Semen collected using electro-ejaculation technique was cooled at 5°C in soy lecithin extender containing 0%, 1%, 2%, 4% and 8% (vol/vol) honey. Semen from the same bulls was frozen at -196°C in the extender containing 0%, 1% and 2% (vol/vol) honey. Semen quality parameters including percentage of total motility, progressive motility, and viability, were assessed on day 1, 3 and 5 post cooling, and on day 7 post freezing. The results showed that the percentage of total and progressive motility remained higher in the extender containing 2% honey for up to 3 days of chilling compared with the extender containing 8% honey and control. Adding honey to frozen sperm, on the other hand, had no effect. Therefore, this study suggests that 2% honey supplemented with soy lecithin extender can be used as an additional source of energy to improve sperm motility in the cool preservation of fighting bull semen.

Keywords: Honey, Soy lecithin extender, Cooled semen, Frozen semen, Fighting bull

[#]Corresponding author

E-mail address: wipawee.s@rmutsv.ac.th

J. Mahanakorn Vet. Med. 2022 17(2): 255-263.

Introduction

Fighting bull or southern native cattle (*Bos indicus*) has been a way of life for a long time and exhibits the uniqueness of the southern Thailand and its local people. Although farmers usually breed their bulls naturally, semen cryopreservation and artificial insemination can help increase the population of domestic animals thereby reducing expenses, especially those related to transportation of bulls. It also helps in conserving genetic component of the animal with superior characteristics for a long period of time (Yoshida, 2000; Barbas and Mascarenhas, 2009). Nevertheless, detrimental effects can partly occur to spermatozoa during the preservation process (Watson, 2000).

Therefore, semen extender solution contains various essential components, and each individual component plays an important role in protecting the spermatozoa during the preservation process. Integral components of semen extenders used for cryopreservation include buffer, cryoprotectants, antibiotics and nutrients (Holt, 2000; Purdy, 2006). Cryoprotectants made from plants like soybean lecithin has been widely used instead of egg yolk to prevent sperm hazards against freezing in cryopreservation process of various animal species in recent years (Aires *et al*., 2003; El-Sisy *et al*., 2016). For the reasons that it provides steady quality, convenience for preparation and reduces risk of bacterial contamination (El-Sisy *et al*., 2016; Layek *et al*., 2016).

Honey is a well-known natural antioxidant with antimicrobial properties in addition to several benefits on various body functions (Bogdanov, 1997; Bogdanov *et al*., 2008). It comprises of high amounts of single molecule sugars and various nutrients, such as vitamins, minerals, and amino acids (Fuller, 2004). Owing to its different properties, previous studies have reported protective effects of added honey into freezing semen media on spermatozoa of goat (Olayemi *et al*., 2011), rabbit (El-Sherbiny, 2013), bull (Yimer *et al*., 2015), buffalo (El-Nattat *et al*., 2016) and stallion (El-Sheshtawy *et al*., 2016). However, the prior studies were all conducted using egg yolk-based semen extender. Thus, the present study aimed to determine the effects of supplementing different concentrations of honey into soy lecithin-based semen extender on cooling and freezing of fighting bull spermatozoa.

Materials and Methods

Animals

Three native fighting bulls from southern Thailand age ranged from two to six years were used for semen collection. Ethical permission for using animal was approved by local institutional animal ethics committee (IAC 03-10-61). All bulls were performed general physical examination and blood check, which were all healthy and without any abnormalities in the reproductive system including brucellosis.

Semen collection and initial evaluation

Six semen samples were collected from each bull once a month using an electro-ejaculation technique (Yimer *et al*., 2011). Immediately following collection, the semen samples were evaluated for volume in graduated tubes, progressive motility using Computer Assisted Semen Analyzer (CASA, IVOS System, Hamilton Thorne Inc., USA), and concentration using a hemocytometer. Only semen samples with more than 70% progressively motile sperm were used for the experiments (Thun *et al*., 2002).

Preservation of semen by cooling method

Immediately following semen collection, fresh semen was diluted with commercial prepared soy lecithin extender (AndroMed®, Minitube, Germany) that had been warmed to 38°C (Aires *et al*., 2003). The diluted semen was

divided into 4 treatment groups by adding 1%, 2%, 4% and 8% of honey (Doi Kham® 100% Honey, Bangkok, Thailand) in semen extender. The final sperm concentration was adjusted to $60x10^6$ spermatozoa/ml. Semen diluted with soy lecithin extender and devoid of honey was used as controls. After dilution, the semen samples of each group were split into 1.5 ml microcentrifuge tubes (Kirgen, Brookfield, USA) followed by storage of the tubes at 5°C (Borges-Silva *et al.*, 2016) for five days. Data collection of the semen parameters was done on day 1, 3 and 5 post semen cooling.

Preservation of semen by freezing method

For the freezing semen experiment, semen sample was diluted with AndroMed® containing honey at 1% and 2% with final concentration of 20 x 10^6 spermatozoa/ml (Bumroongthai *et al*., 2011), while the diluted semen sample with no honey supplementation was kept as a control. Each diluted sample was packed into 0.25 ml straws (IMV, L'Aigle, France) and sealed. The straws were cooled at 4°C for 4 h. The straws were then placed in a horizontal position 5 cm above the liquid nitrogen level (-120°C) for 15 minutes and plunged into liquid nitrogen (-196°C) for storage (Bumroongthai *et al*., 2011) for seven days. Data collection of the semen parameters was done on day 7 post semen freezing.

Assessment of semen quality parameters

Cooled semen samples were warmed and evaluated at 37°C on day 1, 3 and 5 post cooling. For frozen semen samples, the straws were thawed at 37°C for 45 seconds and evaluated on day 7 post freezing. Semen quality parameters including sperm total motility, progressive motility and viability were assessed. Total and progressive motility were analyzed using a computer assisted semen analyzer (CASA). Sperm viability was assessed using eosin-nigrosin stain technique (Felipe-Pérez *et al*., 2008). Each experimental group was evaluated in triplicates.

Statistical analysis

The statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS; Version 16, Chicago, Illinois, USA). To determine the significant difference in all the parameters among all groups, one-way analysis of variance (ANOVA) followed by Bonferroni test was applied. Data are presented as mean ± standard deviation (SD), and the P value of <0.05 were statistically significant.

Results and Discussion

Effect of honey in soy lecithin extender on post-thawing fighting bull chilled spermatozoa

The effect of honey on cooled semen quality is shown in Table 1. The total motility of sperm in the semen solution containing 1% honey and containing 2% honey was significantly higher than the group that contained 8% honey on day 1 of semen cooling ($P<0.05$). In the meantime, only the solution containing 2% honey demonstrated higher total motility than 8% honey and the control group on day 3 of semen cooling ($P<0.05$). In terms of progressive motility of the spermatozoa, the semen solution

containing 2% honey solution showed significantly higher progressive motility than the semen solution containing 8% honey on day 1 and 3 post chilling ($P<0.05$). In addition, the solution containing 2% honey demonstrated significantly higher progressive motility compared to the control group on day 3 of semen cooling ($P<0.05$). This result is consistent with that of studies conducted in buffalo bulls which demonstrated that honey added at 1% to 2% to egg yolk and milk semen extender were associated with high post chilled motility rate (El-Nattat *et al*., 2016; Kandiel and Elkhawagah, 2017). It has been stated that sugars, especially glucose and fructose, act as extracellular non-penetrating cryoprotectants, and provide the main energy source for metabolic processes of spermatozoa (Gil *et al*., 2010; Jerez-Ebensperger *et al*., 2015). This enhancement of sperm motility proves that honey could be the source of energy for movement and a cryoprotectant of spermatozoa (Banday *et al*., 2017).

It has been evidenced that honey is a potent antioxidant in reducing oxidative stress of different body cells including sperm (Al-Waili, 2004; Erejuwa *et al*., 2012). Honey also possesses antimicrobial properties by inhibiting the cytotoxic and genotoxic risks in vivo, and can protect sperm from abnormalities (Zoheir *et al*., 2015). The study in buffalo bulls reported that 0.2% honey could replace the use of antibiotics in extender, and improved the post-thaw motility and plasma membrane integrity of spermatozoa (Nasreen *et al*., 2020). Therefore, these could be additional mechanisms by which the in vitro addition of honey at 2% to soybean lecithin extender improved sperm quality up to 3 days post chilled compared to non-honey treated extenders in this study.

Diminishing motility and viability of spermatozoa in the 8% honey treatment group since day 1 of semen cooling were observed in this study ($P<0.05$) (Table 1). High concentrations of honey above 5% have harmful and toxic effects on sperm during chilling process (Olayemi *et al*., 2011; Yimer *et al*., 2015). Due to the high osmotic property of honey (French *et al*., 2005), supplementing excessive concentrations of honey in semen solution may lead to unbalanced osmotic pressure between the external and internal sperm cells. Therefore, the sperm damage and lose its function during cryopreservation.

Effect of honey in soy lecithin extenders on post-thawing fighting bull cryopreserved spermatozoa

The data showed that total motility, progressive motility and viability of spermatozoa in the semen solution containing 1% and 2% honey were significantly lower than the control post freezing ($P<0.05$) (Table 2). Some studies have asserted the defensive effects of honey addition on post-cryopreservation semen quality (Fakhrildin and Alsaadi, 2014; Yimer *et al*., 2015; El-Sheshtawy *et al*., 2016). However, several animal studies including this did not observe the protective effects of honey against freezing semen process (Kandiel and Elkhawagah, 2017;

Table 1 Parameters of bull spermatozoa quality on day 1, 3 and 5 post semen cooling with honey supplemented in soy lecithin extender.

Day	Parameters	Control	1% Honey	2% Honey	4% Honey	8% Honey
1	TM (%)	45.23±7.46^{ab}	45.57±10.15^{a}	48.31±11.38^{a}	38.14±11.42^{ab}	31.45±9.06^{b}
	PM (%)	36.23±6.88^{ab}	38.73±10.51^{ab}	41.18±10.80^{a}	29.99±12.74^{ab}	25.48±8.01^{b}
	Viability (%)	42.78±7.49^{a}	44.33±10.22^{a}	45.78±9.47^{a}	34.44±7.79^{ab}	28.33±7.53^{b}
3	TM (%)	21.77±7.94^{b}	32.70±8.33^{ab}	37.47±8.64^{a}	24.61±13.00^{ab}	20.23±12.56^{bc}
	PM (%)	15.47±7.77^{bc}	26.13±5.62^{ab}	29.21±9.05^{a}	18.08±11.38^{abc}	13.04±8.67^{cd}
	Viability (%)	18.00±7.90^{a}	23.56±11.90^{a}	27.89±8.03^{a}	18.56±11.38^{a}	14.44±9.12^{a}
5	TM (%)	4.72±5.08^{a}	6.40±6.65^{a}	9.47±13.77^{a}	5.08±6.33^{a}	2.08±2.75^{a}
	PM (%)	2.88±3.30^{a}	3.91±4.95^{a}	6.79±10.86^{a}	2.60±3.65^{a}	1.06±1.32^{a}
	Viability (%)	2.33±5.38^{a}	2.67±5.85^{a}	5.67±10.44^{a}	2.00±4.24^{a}	0.00±0.00^{a}

Note: TM: Total motility; PM: Progressive motility. Mean ± SD with different superscripts are significantly different between groups on the same row at P<0.05.

Table 2 Parameters of bull spermatozoa quality post freezing with honey supplemented in soy lecithin extender.

Parameter	Control	1% Honey	2% Honey
TM (%)	51.11±3.17^{a}	34.07±3.31^{b}	33.68±3.23^{b}
PM (%)	46.60±2.34^{a}	29.45±3.25^{b}	27.32±2.85^{b}
Viability (%)	29.50±2.33^{a}	19.50±2.79^{b}	18.17±2.81^{b}

Note: TM: Total motility; PM: Progressive motility. Mean ± SD with different superscripts are significantly different between groups on the same row at P<0.05.

Malik *et al*., 2019). It has been observed that honey from dissimilar origin had different physicochemical property and microbiological quality (Rosiak *et al*., 2021). Therefore, the reasons could be the differences in animal species and extender condition.

Conclusion

The present study determined that the addition of honey to soy lecithin semen extender enhanced post-chilling total and progressive motility of fighting bull spermatozoa. However, this effect was found to be dependent on the ratio of honey in the extender. The concentration of honey at 2% was discovered to improve post-chilling semen quality, whereas higher concentrations can have adverse effects on sperm motility and viability. However, this study did not detect any defensive effects of honey in the freezing semen experiment. Further

studies are required for evaluation of in vivo potency of honey supplementing semen extender in animals.

Acknowledgment

The authors are grateful for the research funding provided by Rajamangala University of Technology Srivijaya, Thailand. The authors also thank the students and staffs at Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya for their technical assistance during the study.

References

Aires, V.A., Hinsch, K.D., Mueller-Schloesser, F., Bogner, K., Mueller-Schloesser, S. and Hinsch, E. 2003. In vitro and in vivo comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of bovine semen. Theriogenology 60(2): 269-279.

Al-Waili, N.S. 2004. Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. Journal of Medicinal Food 7(2): 210-222.

Banday, M.N., Lone, F.A., Rasool, F., Rather, H.A. and Rather, M.A. 2017. Does natural honey act as an alternative to antibiotics in the semen extender for cryopreservation of crossbred ram semen? Iranian Journal of Veterinary Research 18(4): 258-263.

Barbas, J.P., and Mascarenhas, R.D. 2009. Cryopreservation of domestic animal sperm cells. Cell and Tissue Banking 10(1): 49-62.

Bogdanov, S. 1997. Nature and origin of the antibacterial substances in honey. LWT - Food Science and Technology 30(7): 748-753.

Bogdanov, S., Jurendic, T., Sieber, R. and Gallmann, P. 2008. Honey for nutrition and health: a review. Journal of the American College of Nutrition 27(6): 677-689.

Borges-Silva, J.C., Silva, M.R., Marinho, D.B., Nogueira, E., Sampaio, D.C., Oliveira, L.O.F., Abreu, U.G.P., Mourão, G.B., Sartori, R., 2015. Cooled semen for fixed-time artificial insemination in beef cattle. Reproduction, Fertility and Development 28(7): 1004-1008.

Bumroongthai, K., Banmairuroy, P., Pisamai, S., Singlor, J., Tummaruk, P., 2012. Cryopreservation of Bull Semen by Using Egg Yolk Based Extender Compared with Soya Bean Extract Based Extender. KKU Veterinary Journal 22(1): 51-61.

El-Nattat, W.S., El-Sheshtawy, R.I., El-Batawy, K.A., Shahba, M.I. and El-Seadawy, I.E. 2016. Preservability of buffalo bull semen in tris-citrate extender enriched with bee's honey. Journal of Innovations in Pharmaceutical and Biological Sciences 3(1): 180-185.

El-Sherbiny, A. 2013. Effect of bee honey-included egg yolk based extenders on motility, viability and fertilizing ability of frozen rabbit semen. Egyptian Journal of Rabbit Science 23(2):137-148.

El-Sheshtawy, R.I., El-Badry, D.A., El-Sisy, G.A., El-Nattat, W.S. and Abo Almaaty, A.M. 2016. Natural honey as a cryoprotectant to improve Arab stallion post-thawing sperm parameters. Asian Pacific Journal of Reproduction 5(4): 331-334.

El-Sisy, G.A., El-Nattat, W.S., El-Sheshtawy, R.I. and Abo El-Maaty, A.M. 2016. Substitution of egg yolk with different concentrations of soybean lecithin in tris-based extender during bulls' semen preservability. Asian Pacific Journal of Reproduction 5(6): 514-518.

Erejuwa, O.O., Sulaiman, S.A. and Ab Wahab, M.S. 2012. Honey: a novel antioxidant. Molecules 17(4): 4400-4423.

Fakhrildin, M.B. and Alsaadi, R.A. 2014. Honey supplementation to semen-freezing medium improves human sperm parameters post-thawing. Journal of Family and Reproductive Health 8(1):27-31.

Felipe-Pérez, Y.E., Juárez-Mosqueda, M., Hernández-González, E. and Valencia, J. 2008. Viability of fresh and frozen bull sperm compared by two staining techniques. Acta Veterinaria Brasilica 2(4): 123-130.

French, V.M., Cooper, R.A. and Molan, P.C. 2005. The antibacterial activity of honey against coagulase-negative staphylococci. Journal of Antimicrobial Chemotherapy 56(1): 228-131.

Fuller, B.J. 2004. Cryoprotectants: the essential antifreezes to protect life in the frozen state. CryoLetters 25(6): 375-388.

Gil, L., Mascaró, F., Mur, P., Gale, I., Silva, A., González, N., Malo, C. and Cano, R. 2010. Freezing ram semen: The effect of combination of soya and rosemary essences as a freezing extender on post-thaw sperm motility. Reproduction in Domestic Animals 45: 91.

Holt, W. 2000. Basic aspects of frozen storage of semen. Animal Reproduction Science 62(1-3): 3-22.

Jerez-Ebensperger, R.A., Luño, V., Olaciregui, M., González, N., de Blas, I. and Gil, L. 2015. Effect of pasteurized egg yolk and rosemary honey supplementation on quality of cryopreserved ram semen. Small Ruminant Research 130: 153-6.

Kandiel, M.M.M. and Elkhawagah, A.R.M. 2017. Effect of honey supplementation on Egyptian buffalo semen. Animal Reproduction 14(4): 1103-1109.

Layek, S.S., Mohanty, T.K., Kumaresan, A. and Parks, J.E. 2016. Cryopreservation of bull semen: evolution from egg yolk based to soybean based extenders. Animal Reproduction Science 172: 1-9.

Malik, A., Indah, A., Irwan Zakir, M., Sakiman, S. and Nugroho, S. 2019. Cryopresevative effect of adding a honey solution to native chicken spermatozoa. Advances in Animal and Veterinary Sciences 7(4): 261-4.

Nasreen, S., Awan, M.A., ul-Husna, A., Rakha, B.A., Ansari, M.S., Holt, W. and Akhter, S. 2020. Honey as an alternative to antibiotics for cryopreservation of Nili-Ravi buffalo bull spermatozoa. Biopreservation and Biobanking 18(1): 25-32.

Olayemi, F.O., Adeniji, D.A. and Oyeyemi, M.O. 2011. Evaluation of sperm motility and viability in honey included egg yolk based extenders. Global Veterinaria 7(1): 19-21.

Purdy, P.H. 2006. A review on goat sperm cryopreservation. Small Ruminant Research 63(3): 215-225.

Rosiak, E., Madras-Majewska, B., Teper, D., Łepecka, A., Zieliǹska, D. 2021. Cluster analysis classification of honey from two different climatic zones based on selected physicochemical and of microbiological parameters. Molecules 26(8): 2361.

Thun, R., Hurtado, M. and Janett, F. 2002. Comparison of Biociphos-Plus® and TRIS-egg yolk extender for cryopreservation of bull semen. Theriogenology 57(3): 1087-1094.

Watson, P.F. 2000. The causes of reduced fertility with cryopreserved semen. Animal Reproduction Science 60-61: 481-492.

Yimer, N., Haron, A.W., Saharee, A.A., Yap, K.C., Ganesamurthi, P. and Fahmi, M. 2011. Trans-scrotal ultrasonography and breeding soundness evaluation of bulls in a herd of dairy and beef cattle with poor reproductive performance. Pertanika Journal of Tropical Agricultural Science 34(2): 217-228.

Yimer, N., Muhammad, N., Sarsaifi, K., Rosnina, Y., Haron, A.W., A.M, K. and Kaka, A. 2015. Effect of honey supplementation into Tris extender on cryopreservation of bull spermatozoa. Malaysian Journal of Animal Sciences 18(2): 47-54.

Yoshida, M. 2000. Conservation of sperms: current status and new trends. Animal Reproduction Science 60-61: 349-355.

Zoheir, K.M.A., Harisa, G.I., Abo-Salem, O.M. and Ahmad, S.F. 2015. Honey bee is a potential antioxidant against cyclophosphamide-induced genotoxicity in albino male mice. Pakistan Journal of Pharmaceutical Sciences 28(3): 973-981.