Effect of Various Coconut (*Cocos nucifera*) Milk Concentrations Instead of Egg Yolk on Freezability and Fertility of Frozen Holstein Bull Spermatozoa

EL-SHAMAA, Ibrahim

Animal Production Department, Faculty of Agriculture, Kafrelsheikh University | Laboratory of Reproductive Physiology and Biotechnology, Department of Animal and Marine Bioresource Sciences, Faculty of Agriculture, Kyushu University

IBRAHIM, Mostafa

Animal Production Department, Faculty of Agriculture, Kafrelsheikh University | Laboratory of Reproductive Physiology and Biotechnology, Department of Animal and Marine Bioresource Sciences, Faculty of Agriculture, Kyushu University

EL-SHARAWY, Mohamed

Animal Production Department, Faculty of Agriculture, Kafrelsheikh University | Laboratory of Reproductive Physiology and Biotechnology, Department of Animal and Marine Bioresource Sciences, Faculty of Agriculture, Kyushu University

MAHMOUD, Raed

Animal Production Department, Faculty of Agriculture, Kafrelsheikh University | Laboratory of Reproductive Physiology and Biotechnology, Department of Animal and Marine Bioresource Sciences, Faculty of Agriculture, Kyushu University

他

<https://doi.org/10.5109/1955394>

出版情報：九州大学大学院農学研究院紀要. 63 (2), pp.265-270, 2018-09-01. Faculty of Agriculture, Kyushu University

バージョン：

権利関係：

Effect of Various Coconut (*Cocos nucifera*) Milk Concentrations Instead of Egg Yolk on Freezability and Fertility of Frozen Holstein Bull Spermatozoa

Ibrahim EL-SHAMAA^{1*}, Mostafa IBRAHIM¹, Mohamed EL-SHARAWY¹, Raed MAHMOUD¹,
Kaiyu KUBOTA², Nobuhiku YAMAUCHI³ and El-Shenawy El-SIEFY⁴

Laboratory of Reproductive Physiology and Biotechnology, Department of Animal and Marine Bioresource Sciences,
Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan
(Received April 4, 2018 and accepted May 8, 2018)

The study was carried out to investigate if the substitution of egg yolk (EY) with various coconut (*Cocos nucifera*) milk concentrations in extenders can improve the freezability and fertility of frozen Holstein bull spermatozoa. Semen from five Holstein bulls was collected twice weekly and ejaculates with 75% progressive motility and more 85% normal sperm morphology prior to cryopreservation were pooled in order to have sufficient semen for a replicate and to eliminate the bull effect. Five extenders were used. Tris 20% egg yolk extender with 7% glycerol as a control, and substitution of whole egg yolk with 5, 10, 15, and 20% coconut milk. Semen was diluted to 80 million sperm/ml packaged into 0.25ml straws, cooled held at 5°C for 4 h, and then frozen in liquid nitrogen and stored at –196°C until for artificial insemination. Sperm progressive motility, live sperm, sperm abnormality, intact sperm acrosome and plasma membrane integrity were evaluated post dilution, post-equilibration and post frozen-thawed processes. The results revealed that 5% coconut milk extender was more effective in preservation of progressive motility, live sperm, sperm abnormality, intact sperm acrosome and plasma membrane integrity of Holstein spermatozoa than whole egg yolk extender and other coconut milk extenders. Fertility rates were higher in 5% and 10% coconut milk extenders compared with whole egg yolk extender (65% and 55% vs 45%, respectively) and other 15% and 20% coconut milk extenders (45% and 40%, respectively). It was concluded that 5% coconut milk extender improved the freezability and fertility of Holstein bull spermatozoa.

Key words: Holstein Bull Semen, Coconut Milk, Freezing, Fertility

INTRODUCTION

Sperm cryopreservation is the most efficient method for storing bull spermatozoa for long period even though their fertilizing ability is still, lower than that of fresh or liquid-preserved semen (Hammerstedt *et al.*, 1990). Successful semen cryopreservation depends on the use of suitable extender, cryoprotectant and the proper cooling/warming process (Fiser, 1991). Mainly, the cryoprotectant plays a major role in this procedure by resisting sudden temperature change, protecting sperm against cold and hot shock damage (Watson, 1999), as well as preventing ice formation during freezing-thawing process. However, Morris *et al.* (2007) reported that intracellular ice formation does not induce significant cryodamage, and limited water was observed in sperm. Egg yolk is frequently used as a cryoprotectant agent in mammalian semen diluted and showed to be highly effective for the maintenance of sperm fertility stages (Sansone *et al.*, 2000; Garde *et al.*, 2003). It is believed that the beneficial effect of EY during cryopreservation process can be attributed to phospholipids, cholesterol low density

lipoproteins which protecting sperm against cold shock (Moussa *et al.*, 2002; El-Sharawy *et al.*, 2012^{a,b}). Norman (1962) is the first used coconut milk as part of extender for semen and who added also coconut milk had the advantage of keeping livability of fresh semen. In goat semen, Melo and Nunes (1991) reported that semen extended in coconut milk led to give appreciable sperm cell motility and fertility with acceptable conception rate post-breeding. Coconut milk is contained energy compounds such as sugars (glucose, fructose, lactose, and sucrose), fatty acids, proteins and amino acids (glutamic acid, glutamine, alanine, arginine, lysine, leucine, proline, etc), minerals and trace elements (Na⁺, Ca²⁺, K²⁺, Mg²⁺, Cl⁻, Fe³⁺, P, N₂, Co²⁺, Zn²⁺, PO₄⁻, HCO₃⁻ – etc), vitamins, antioxidant enzymes (catalase, peroxidase), phospholipids, and phytohormones (cytokinins) for example kinetin reputed to have ant stress, anti-ageing, anti-carcinogenic and anti-thrombotic effects (Abara *et al.*, 2007; DebMandal and Mandal, 2011; Solangi and Iqbal, 2011). Sule *et al.* (2007) reported that coconut milk has been employed in various proportions and in varying combinations in semen extenders for storage (hours to days) under controlled and ambient conditions. However, to our knowledge, no report has evaluated the effect of coconut milk in extender on freezability of bull semen under –196°C in liquid nitrogen for long storing.

Therefore, the present study was carried out to evaluate and compare the effect of substitution of EY with coconut milk on bull semen cryopreservation and on subsequent fertilization.

¹ Animal Production Department, Faculty of Agriculture, Kafrelsheikh University, 33516, Egypt

² Hitachi City Community Obstetrics and Gynecology, Tokyo Medical University, Shinjuku-ku 160–0023

³ Department of Animal and Marine Bioresource Sciences, Graduate School, Kyushu University, Fukuoka 812–8581, Japan

⁴ Biotechnology Research Department, Animal Production Research Institute, Agriculture Research Center, Egypt

* Correspondence author (E-mail: elshamaa2008@yahoo.com)

MATERIALS AND METHODS

Experimental animals:

This study was conducted for a periods of 8 weeks. Five adult Holstein bulls, aged (3–5 years) with clinically normal reproductive tract and kept at the International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture. The bulls included herein were regular semen donors with satisfactory semen quality and having good fertility records.

Semen collection:

Semen was collected twice weekly, using an artificial vagina, from each bull and immediately held in a water bath adjusted at 37°C and evaluated for sperm motility. Ejaculates showing at least 75% sperm motility and more than 85% normal spermatozoa were selected for further processing. On each collection day, good ejaculates were pooled and divided into 5 parts; the first was diluted with Tris–20% EY (control), while the other four parts were diluted with Tris extender without EY supplemented with coconut milk at levels of 5, 10, 15 and 20%, respectively.

Preparation of semen extenders:

Tris-based extender: 3.025 g Tris– (hydroxymethyl-aminomethane), 1.675 g citric acid, 0.75 g glucose and 7.0% glycerol, 20% egg, 0.25 g lincomycin and 0.05 g streptomycin and completed with bi-distilled water up to 100 ml.

Coconut milk: The preparation of coconut milk extender followed a simple but aseptic procedure. The meat of freshly harvested coconut (*Cocos nucifera*) was thoroughly blended and collected in a 250 ml conical flask. The water from the coconut was added to the blend and the mixture allowed standing for about 1 h. Thereafter, the mixture was wrapped in a heat sterilized white cloth and tightly squeezed to express the milk. The milk was filtered through sterilized white clothes thrice to get rid of all residues and the liquid was collected in a sterilized flask. The whole 20% EY in Tris-based extender was replaced by different concentrations of 5, 10, 15, and 20% coconut milk extraction.

Extension and freezing semen:

Each pooled semen sample was split into five aliquots and extended at 37°C with one of five experimental extenders to achieve the final concentration of 80 million spermatozoa per ml of the extended semen. Immediately after extension, the percentage of progressive motility, live sperm, sperm abnormality, acrosome and plasma membrane integrity were recorded. Then, extended semen was maintained in a refrigerator at 5°C for 4 h for equilibration. The samples were filled in 0.25 ml French straws with the help of an automatic suction machine. Subsequently, these straws were placed 4 cm above the liquid nitrogen surface where the temperature was approximately –120°C. After 10 min all straws were

immersed directly into liquid nitrogen at –196°C for freezing. Also, the percentages of progressive motility, live sperm, sperm abnormality, intact acrosome integrity and plasma membrane integrity were evaluated post-equilibration and post –thawing processes.

Thawing frozen semen:

The straws containing the EY, 5%, 10%, 15% and 20% coconut milk extenders were plunged directly into water bath at 37°C for 30 Sec, then wiped with absorbent paper. The ends were then cut and the contents emptied into a plain tube maintained at 37°C and were analyzed 10 min later. Progressive motility was estimated according to Melrose and Laing (1970) live and abnormalities were estimated according to Hancock (1951) acrosome and plasma membrane integrity were determined according to Kovacs and Foote (1992) and Jeyendran *et al.* (1984) respectively.

Fertility trail:

A total of 100 Holstein cows were artificial inseminated with random frozen doses from five various extenders (20 cow each). Each cow was inseminated with a single straw 8–14 after start of estrus behavior. Using rectovaginal technique and the universal insemination gun, the frozen–thawed semen was deposited in the uterine body just next to the anterior end of the cervix. Conception rate was calculated on the basis of pregnancies confirmed by rectal palpation 45–60 day after insemination.

Statistical analysis

Data were statistically analyzed by the methods of analysis of variance according to model procedures of SPSS (2013). Duncan Multiple Range Test was used to test the differences among means (Duncan, 1955).

RESULTS

The effect of different concentrations of coconut milk in Tris–extender and Tris–20% egg on frozen Holstein sperm quality parameters are shown in Tables 1, 2, 3, 4 and 5.

The progressive motility of Holstein spermatozoa in 5% coconut milk extender was equal with that in control Tris–egg yolk extender post dilution (72.1–72.3%, respectively) but it was significantly ($P < 0.05$) higher during post equilibration and post frozen–thawed processes ($66 \pm 0.69\%$ and $57.3 \pm 0.99\%$ vs $60.5 \pm 1.19\%$ and $48.5 \pm 1.21\%$ respectively (Table 1). In addition, the progressive motility of spermatozoa in 10% and 15% coconut milk extenders were higher after equilibration and post frozen–thawed processes compared to that in control Tris–egg yolk extender and 20% coconut milk extender. The 20% coconut milk showed the lower progressive sperm motility during post–dilution, equilibration and thawing processes than all other extenders.

The percentage of live Holstein spermatozoa was improved in 5%, 10%, and 15% coconut milk extenders post equilibration and post frozen–thawed processes

compared to control and 20% coconut milk extenders (Table 2). In addition, the increasing coconut milk to 20% led to decrease the percentage of sperm livability post three stages of cryopreservation compared to other extenders.

The percentage of sperm abnormality was lower only in semen extended with 5% coconut milk extender compared to control and other coconut milk extenders dur-

ing post-dilution, post-equilibration and post frozen-thawed processes (Table 3). The highest sperm abnormality was observed in 10%, 15% and 20% coconut milk extenders compared to 5% coconut milk and control (Tris-egg yolk extenders).

The percentage of sperm plasma membrane integrity in 5% coconut milk extender was significantly ($P<0.05$) higher than control and other coconut milk extenders

Table 1. Effect of Tris-EY and coconut milk extenders on progressive motility (%) of Holstein bull spermatozoa at different stages of cryopreservation. (Mean $\pm$ S.E)

Item	Tris-EY* (Control)	Tris-Coconut milk levels (%)			
		5	10	15	20
Post dilution	72.3 ^a $\pm$ 0.77	72.1 ^a $\pm$ 0.79	68.0 ^b $\pm$ 1.11	67.5 ^b $\pm$ 0.92	63.0 ^c $\pm$ 0.67
Post equilibration	60.5 ^b $\pm$ 1.19	66 ^a $\pm$ 0.69	62.3 ^b $\pm$ 0.99	60.8 ^b $\pm$ 1.32	54.0 ^c $\pm$ 1.24
Post thawing	48.5 ^c $\pm$ 1.21	57.3 ^a $\pm$ 0.99	53.8 ^b $\pm$ 0.99	49.8 ^c $\pm$ 1.33	42.5 ^d $\pm$ 1.23

*Tris-EY: Tris Egg Yolk Extender

a, b, c and d: the different superscripts in the same row are significant at ($P<0.05$).

Table 2. Effect of Tris-EY and coconut milk extenders on live spermatozoa (%) of Holstein bull spermatozoa at different stages of cryopreservation. (Mean $\pm$ S.E)

Item	Tris-EY* (Control)	Tris-Coconut milk levels (%)			
		5	10	15	20
Post dilution	76.3 ^a $\pm$ 0.81	76.3 ^a $\pm$ 0.69	73.3 ^b $\pm$ 1.03	71.1 ^b $\pm$ 1.06	67.0 ^c $\pm$ 0.73
Post equilibration	64.9 ^b $\pm$ 1.03	69.9 ^a $\pm$ 0.80	66.1 ^b $\pm$ 1.19	65.2 ^b $\pm$ 1.29	57.4 ^c $\pm$ 1.45
Post thawing	52.5 ^c $\pm$ 1.01	61.5 ^a $\pm$ 1.00	57.7 ^b $\pm$ 0.89	53.9 ^c $\pm$ 1.46	47.5 ^d $\pm$ 1.24

*Tris-EY: Tris Egg Yolk Extender.

a, b, c and d: the different superscripts in the same row are significant at ($P<0.05$).

Table 3. Effect of Tris-EY and coconut milk extenders on abnormality (%) of Holstein bulls spermatozoa at different stages of cryopreservation. (Mean $\pm$ S.E)

Item	Tris-EY* (Control)	Tris-Coconut milk levels (%)			
		5	10	15	20
Post dilution	7.1 ^c $\pm$ 0.14	7.2 ^{bc} $\pm$ 0.14	7.8 ^a $\pm$ 0.12	7.5 ^{abc} $\pm$ 0.17	7.6 ^{ab} $\pm$ 0.14
Post equilibration	8.0 ^c $\pm$ 0.12	8.8 ^c $\pm$ 0.20	9.0 ^{ab} $\pm$ 0.18	9.1 ^{ab} $\pm$ 0.26	9.5 ^a $\pm$ 0.27
Post thawing	10.2 ^c $\pm$ 0.23	10.9 ^{bc} $\pm$ 0.26	11.3 ^{ab} $\pm$ 0.19	11.4 ^{ab} $\pm$ 0.24	12.0 ^a $\pm$ 0.32

*Tris-EY: Tris Egg Yolk Extender.

a, b and c: the different superscripts in the same row are significant at ($P<0.05$).

Table 4. Effect of Tris-EY and coconut milk extenders on plasma membrane integrity (%) (Mean $\pm$ S.E) of Holstein bull spermatozoa, at different stages of cryopreservation

Item	Tris-EY* (Control)	Tris-Coconut milk levels (%)			
		5	10	15	20
Post dilution	74.8 ^a $\pm$ 0.63	73.7 ^a $\pm$ 0.72	70.6 ^b $\pm$ 1.02	68.7 ^b $\pm$ 0.86	65.0 ^c $\pm$ 0.55
Post equilibration	63.9 ^b $\pm$ 0.80	67.9 ^a $\pm$ 0.89	63.2 ^b $\pm$ 0.95	63.4 ^b $\pm$ 1.11	57.2 ^c $\pm$ 1.29
Post thawing	51.5 ^c $\pm$ 0.94	59.4 ^a $\pm$ 1.03	56.0 ^b $\pm$ 0.82	52.4 ^c $\pm$ 1.30	45.45 ^d $\pm$ 1.27

*Tris-EY: Tris Egg Yolk Extender.

a, b, c and d: the different superscripts in the same row are significant at ($P<0.05$).

Table 5. Effect of Tris-EY and coconut milk extenders on acrosome integrity (%) (Mean $\pm$ S.E) of Holstein bull spermatozoa, at different stages of cryopreservation

Item	Tris-EY* (Control)	Tris-Coconut milk levels (%)			
		5	10	15	20
Post dilution	73.65 ^{ab} $\pm$ 0.58	72.31 ^a $\pm$ 0.76	69.20 ^b $\pm$ 0.99	67.95 ^b $\pm$ 0.92	63.80 ^c $\pm$ 0.76
Post equilibration	63.80 ^{ab} $\pm$ 1.02	66.75 ^a $\pm$ 0.90	61.30 ^b $\pm$ 1.07	61.70 ^b $\pm$ 1.20	56.35 ^c $\pm$ 1.15
Post thawing	51.30 ^c $\pm$ 1.06	58.45 ^a $\pm$ 0.94	54.65 ^b $\pm$ 0.87	50.90 ^c $\pm$ 1.45	44.90 ^d $\pm$ 1.29

*Tris-EY: Tris Egg Yolk Extender.

a, b, c and d: the different superscripts in the same row are significant at ($P<0.05$).

Table 6. Effect of Tris-EY and coconut milk extenders on conception rate of Holstein

Item	Tris-EY* (Control)	Tris-Coconut milk levels (%)			
		5	10	15	20
Inseminated animals	20	20	20	20	20
Conceived animals	9	13	11	9	8
Conception rate (%)	45	65	55	45	40

*Tris-EY: Tris Egg Yolk Extender.

during equilibration and post -thawing processes (Table 4). In addition, semen extended with 10% and 15% coconut milk extenders improved plasma membrane integrity at post- equilibration and post -thawing processes compared to that in control Tris-egg yolk extender and 20% coconut milk extender. Moreover, increasing coconut milk in semen extended to 20% led to lower ($P<0.05$) plasma membrane integrity compared to control Tris-egg yolk extenders during all stages of cryopreservation. The trend of results observed in intact sperm acrosome (Table 5) was the same with the previous percentage of sperm plasma membrane integrity.

Conception rate:

The effect of different coconut milk extenders and Tris-egg yolk extender on conception rate are shown in Table 6. Semen extended with 5% and 10% coconut milk extenders improved conception rate to 65% and 55%, compared to that in control-Tris egg yolk extender and 15% or 20% coconut milk extender (45% and 45% or 40% respectively).

DISCUSSION

The present findings indicated that using coconut milk in semen extender improved the quality of cryopreserved spermatozoa of Holstein bull and its subsequent fertility. The present results demonstrated coconut milk extenders (except level 20%) provided a more adequate medium to sustain the progressive motility, live sperm, intact plasma membrane and intact acrosome of Holstein spermatozoa cryopreserved as evidenced from its ability to maintain sperm parameters better than the control. This improvement observed on sperm frozen-thawed parameters indicated that coconut milk extenders (5%, 10% and 15%) contained essential constituents such as

sugars, vitamins, minerals and amino acids (Yong *et al.*, 2009; USDA National Nutrient Database, 2015) required for cryosurvival of spermatozoa.

Many authors confirmed that the role of sugars as a source of energy, an osmolyte and a cryoprotectant of sperm survival following cryopreservation process (Yancy, 2005; Purdy, 2006; Naing *et al.*, 2010; Daramola *et al.*, 2016^a). In goat semen, Koshimoto and Mazur (2002) and Aboagla and Terada (2003) suggested that goat spermatozoa readily utilizes sugar for respiration, and these sugars also provide osmotic balance and cryopreservation. The reduction in sperm motility, live sperm, plasma membrane integrity and acrosome integrity in various coconut milk extenders during post dilution process might been caused by an increase in the number of sperm abnormalities.

The protective effect of coconut milk also be linked to its high content of lipids (Yang *et al.*, 2009) which a major component of sperm membrane that is involved in a series of biochemical and functional change ultimately required for fertilization (Brque *et al.*, 2003).

During cryopreservation, significant changes in lipid composition and phospholipids loss in seminal plasma occur (Chakrabarty *et al.*, 2007; Futino *et al.*, 2010) leaving sperm vulnerable to oxidative stress due to of antioxidant protection (Bucak *et al.*, 2010). Therefore, the exogenous phospholipids present in extenders derived from coconut milk possibly replaced some of the sperm membrane phospholipids lost during cryopreservation to maintain the plasma membrane structure and function (Zhang *et al.*, 2009). Moreover, phospholipids play important physiological function in reducing the freezing point, avoiding the formation of large ice crystals and resulting in reduced mechanical damage to sperm membrane (Giraud *et al.*, 2000; Waterhouse *et al.*, 2006; Daramola *et al.*, 2016^b). Furthermore, the protective

effect of coconut milk could also be attributed to its major proteins including essential amino acids which play an important role in cell membrane integrity (Sakanaba *et al.*, 2004; Yang *et al.*, 2009). The cryoprotective effect of amino acids in mammalian spermatozoa during freezing stemmed from their ability to form a layer on the spermatozoa surface, and positively charged molecules combined with the phosphate groups of sperm plasma membrane phospholipids has been observed (Kundu *et al.*, 2001; Atessahin *et al.*, 2008).

In addition, the improved frozen-thawed of Holstein spermatozoa in the present study with 5% coconut milk extender indicated the ability in coconut milk extender to efficiency harness the potassium contained in coconut milk and spermatozoa for survival of the spermatozoa during cryopreservation (Okolie *et al.*, 2011). 20% coconut milk extender was the poorest semen extender probably on coconut milk of its high viscosity which impeded sperm motility (Sule *et al.*, 2007; Okukpe *et al.*, 2012).

The present improvement in cryopreserved spermatozoa was closed with that reported by Daramola *et al.* (2016^{ab}) in buck semen. Conception rate in cow inseminated with semen cryopreserved in extenders containing different coconut milk concentrations (5% and 10%) were higher than control and other 15% or 20% coconut milk extenders (65% and 55% vs 45% and 45% or 40%, respectively) see Table 6. It showed be mentioned that conception rate achieved in the present study (55% to 65%) is satisfactory as compared with the previous studies using freezing and thawing techniques.

Based on the foregoing results, it could be concluded that coconut milk extender process remarkable cryoprotective properties for freezing Holstein bull spermatozoa. Higher frozen-semen quality and conception rate were achieved with the use of 5% and 10% coconut milk extenders as compared to 20% egg yolk semen extender.

AUTHOR CONTRIBUTIONS

- 1) Study conception and design: I. EL-SHAMAA; R. MAHMOUD; E. EL-SIEFY and M. IBRAHIM
- 2) Acquisition of data: R. MAHMOUD; E. EL-SIEFY; I. M. IBRAHIM and I. EL-SHAMAA
- 3) Analysis and interpretation of data: E. EL-SIEFY; M. EL-SHARAWY; N. YAMAUCHI and I. EL-SHAMAA
- 4) Drafting of manuscript: I. EL-SHAMAA; R. MAHMOUD; E. EL-SIEFY; M. EL-SHARAWY; N. YAMAUCHI and K. KUBOTA
- 5) Critical revision: M. EL-SHARAWY; M. IBRAHIM; K. KUBOTA; N. YAMAUCHI and I. EL-SHAMAA

REFERENCES

- Abara, A. E., G. O. Obochi, S. P. Malu, M. Obi-Abang, V. S. Ekam and F. U. Uboh 2007 Effect of caffeine coconut product interactions on induction of microsomal drug-metabolising enzymes in Wistar albino rats. *Nigerian J. Physiological Sci.*, **22**: 75-81
- Abogla E. M. E. and T. Terada 2003 Trehalose-enhanced fluidity of the goat sperm membrane and its protection during freezing. *Biol Reprod*, **69**: 1245-1250
- Atessahin, A., M. N. Bucak, P. B. Tuncer and M. Kizil 2008 Effects of antioxidant activities on microscopic and oxidative parameters of Angora goat semen following the freezing-thawing process. *Small Rumin. Res.*, **77**: 38-44
- Brque, C., P. Surai and J. P. Brillard 2003 Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. *Mol. Reprod. Dev.*, **66**: 314-323
- Bucak, M. N., S. Sarizkan, P. B. Tuncer, F. Sakin, M. Atessahin, R. Kulaksız and M. Cevik 2010 The effect of antioxidants on post-thawed Angora goat (*Capra hircusancryrensis*) sperm parameters, lipid peroxidation and antioxidant activities. *Small Rumin. Res.*, **89**: 24-30
- Chakrabarty, J., D. Banerjee, D. Pal, J. De, A. Ghosh and G. C. Majumder 2007 Shedding off specific lipid constituents from sperm cell membrane during cryopreservation. *Cryobiology* **54**: 27-35
- Daramola, J. O., E. O. Adekunle, O. S. Iyasere, O. E. Oke, T. A. Sorongbe, O. A. Iyanda, A. R. Kehinde, S. P. Aluko, I. O. Olaoye, O. E. Gbadebo, L. I. Falolu, R. A. Ajayi, O. J. Enikannaye and E. D. Osunjaiye 2016^a Effects of coconut milk alone or supplementation with pyridoxine in tris-extendors on viability of buck spermatozoa during vitrification. *Small Rumin. Res.*, **136**: 208-213
- Daramola, J. O. and E. O. Adekunle; O. E. Oke; O. M. Onagbesan; I. K. Oyewusi and J. A. Oyewusi 2016^b Effects of coconut (*Cocos nucifera*) water with or without egg-yolk on viability of cryopreserved buck spermatozoa. *Anim. Reprod., Belo Horizonte*, **13**: 57-62
- DebMandal M. and S. Mandal 2011 Coconut (*Cocos nucifera* L.: Arecaceae): In health promotion and disease prevention. *Asian Pacific Journal of Tropical Medicine*, 241-247
- Duncan, D. B. 1955. Multiple Range and Multiple F. *Test Biometrics*, **11**: 1-42
- El-Sharawy M. E., I. S. El-Shamaa, M. AR. Ibrahim and M. E. El-Seify 2012^a Using of low density lipoproteins and glutamine to improve frozen buffalo bull semen and fertility. *Reprod. Domest. Anim.*, **47**: 444 (Abstract)
- El-Sharawy M. E., I. S. El-Shamaa, M. A. R. Ibrahim, I. M. Abd El-Razek and E. M. El-Seify 2012^b Effect of low density lipoproteins in extender on freezability and fertility of Egyptian Buffalo bull semen. *Animal Science Series D*, Vol. (LV): 114-120
- Fiser, P. S. 1991 Interactions of cooling velocity, warming velocity and glycerol concentration on the survival of frozen-thawed boar sperm. In: Johnson LA, Rath D, eds. *Reproduction in Domestic Animals, Boar Semen Preservation II*. Proceedings of the 2nd International Conference on Boar Semen Preservation. Berlin and Hamburg: Paul Parey Scientific, 123-137
- Futino, D., M. Mendes, W. Matos, R. Mondadori and C. Lucci 2010 Glycerol: methyl-formamide and dimethyl-formamide in canine semen cryopreservation. *Reprod. Domest. Anim.*, **45**: 214-220
- Giraud, M. N., C. Motta, D. Boucher and G. Grizard 2000 Membrane fluidity predicts the outcome of cryopreservation of human spermatozoa. *Hum. Reprod.*, **15**: 2160-2164
- Hammerstedt, R. H., J. K. Graham and J. P. Nolan 1990 Cryopreservation of mammalian sperm: what we ask them to survive. *J. Androl.*, **11**: 73-88
- Hancock, J. I. 1951 *A staining technique for the study of temperature shock in semen*. Nature, Lond., **167**: 223
- Jeyendran, R. S., H. H. Van Der Ven, M. Perez-Pelaez, B. G. Crabo and L. J. D. Zaneveled 1984 Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Reprod. Fertil.*, **70**: 219-228
- Koshimoto, C. and P. Mazur 2002 The effect of the osmolality of sugar-containing media the type of sugar, and the mass and molar concentration of sugar on the survival of frozen-thawed mouse sperm. *Cryobiology* **45**: 80-90
- Kovacs, A. and R. H. foote. 1992. Viability and acrosome staining of bull, boar and rabbit spermatozoa. *Biotechnic and Histochemistry* **67**: 119-124
- Kundu, C. N., K. Das and G. C. Majumder 2001 Effect of amino

- acids on goat cauda epididymal sperm cryopreservation using a chemically defined model system. *Cryobiology* **41**: 21–27
- Melo, A. C. M. and J. F. Nunes 1991 Use of coconut milk and milk-glucose as diluents for frozen goat semen. *Anais IX Congresso-Brasileiro de Reproducao Animal*. Belo Horizonte. Brazil. 22 a 26 de Junho de 1991, II. p. 435
- Melrose, D. R. and J.A. Laing 1970 *The characteristics of normal semen*. Ed. By J. A. laing Baillieretindall and Cassel. London.
- Morris, G. J., K. faszer, J. E. Green, D. Draper, B. W. Grout and F. Fonseca 2007 Rapidly cooled horse spermatozoa loss of viability is due to osmotic imbalance during thawing, not intracellular ice formation. *Theriogenology*, **68**: 804–812
- Moussa M., V. Martinet, A. Trimeche, D. Tainturier and M. Anton 2002 Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen-thawed bull semen. *Theriogenology*, **57**: 1695–1706
- Naing, S. W., H. Wahid, K. Mohd Azam, Y. Rosnina, A. B. Zuki, S. Kazhal, M. M. Bakar, M. Thein, T. Kyaw, M. M. San 2010 Effect of sugars on characteristics of Boer goat semen after cryopreservation. *Anim. Reprod. Sci.*, **122**: 23–28
- Norman, C. J. 1962 Survival and fertility of bovine spermatozoa kept at variable temperature in coconut milk extender. *J. Agric. Sc.*, **59**: 1803–1807
- Okolie, P. N., C. L. Obi and P. O. Uaboi-Egbenni 2011 Fungal spoilage of coconut (*Cocos nucifera* L.) fruits during storage and the growth differential of isolates on selected amino acids and carbohydrates. *Pak. J. Nutr.*, **10**: 965–973
- Okukpe, K. U., A. A. Adeloye, K. O. Adeyemi, O. A. Olatude, V. Ojo and F. E. Sola-Ojo 2012 Effect of extender types on ram semen collected with electroejaculator in a tropical enviroment. *Asian J. Anim. Sci.*, **6**: 249–255
- Purdy P. H. 2006 A review on goat sperm cryopreservation. *Small Rumin. Res.*, **63**: 215–225
- Sakanaba, S., T. Tachibana, N. Ishihara and L. R. Juneja 2004 Antioxidant activity of egg-yolk protein hydrolysates in a linolenic acid oxidation system. *Food Chem.* **86**: 99–103
- Sansone, G., M. F. Nastria and A. Fabbrocini 2000 Storage of buffalo (*Bubalus bubalis*) semen. *Anim. Reprod. Sci.*, **62**: 55–76
- Solangi, A. H. and M. Z. Iqbal 2011 Chemical composition of meat (kernel) and nut water of major coconut (*Cocos nucifera* L.) Cultivers at coastal area of Pakistan. *Int. J. Botany* **43**: 351–363
- SPSS. 2013 SPSS user Guide: Statistics SPSS.
- Sule, W. F, M. O. Oyeyemi and M. O. Akusu 2007 Coconut milk-citrate as extender for West African Dwarf buck spermatozoa at room temperature. *Biokem.*, **19**: 65–73
- USDA, 2015 USDA National Nutrient Database. Coconut meat, raw, (*Cocus nucifera*), fresh, nutrition value per 100 g. <http://www.nutrition-and-you.com/coconut.html>.
- Waterhouse, K. E., P. O. Hofmo, A. Tverdal and R. R. Miller 2006 Within and between breed differences in freezing tolerance and plasma membrane fatty acid composition of boar sperm. *Reprod.*, **131**: 887–894
- Watson, P. F. 1995 Cooling of spermatozoa and fertility capacity. *Reprod. Domest. Anim.*, **31**: 135–140
- Yancey, P. H. 2005 Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.* **208**: 2819–2830
- Yong, J. W., G. Liya, F. Ng. Yan and S. Ng. Tan 2009 The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules* **14**: 5144–5164
- Zhang, S. S., J. H. Hu, Q. W. Li, Z. L. Jiang, X. Y. Zhang 2009 The cryoprotective effects of soybean lecithin on boar spermatozoa quality. *Afr. J. Biotechnol.*, **8**: 6476–6480