INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 1, p. 393-398, 2014

RESEARCH PAPER OPEN ACCESS

Effect of addition of raffinose and trehalose at andromed on post thawed semen characteristics of buffalo spermatozoa

Khadijeh Piri[1*], Jamshid Ghiasi Ghalehkandi[1], Rahim Beheshti[2]

[1]*Department of Animal Science, Shabestar Branch, Islamic Azad University- Shabestar- Iran*

[2]*Department of Veterinary Medicine, Shabestar Branch, Islamic Azad University- Shabestar- Iran*

Key words: Spermatozoa, raffinose, trehalose, buffalo.

http://dx.doi.org/10.12692/ijb/4.1.393-398 Article published on January 11, 2014

Abstract

The main purpose of the current study was to investigate protective effects of raffinose and trehalose on post thawing characteristics of buffalo spermatozoa. A total number of 20 ejaculates were collected using the artificial vagina from four native buffalos. All Ejaculates were evaluated and pooled at 37 °C and had average 70% motile spermatozoa. The semen was diluted by commercial Andromed containing different levels of raffinose or trehalose (25 and 75 mmol) and without any supplement (control). Semen was cooled to 4 °C for a period of 4 h. samples were loaded into 0.05 mL straws contain 20×106 cells/mL, frozen and stored in liquid nitrogen for 2 weeks. Forty second period used to freeze- thawing process at 37 °C. Sperm motility and quality characteristics were determined using microscope and computer evaluation system (CASA) on admission and 6 hrs post thawing. According to the results, there was a significant decrease on post-thawing spermatozoa motility and quality characteristics using different levels of Raffinose and Trehalose containing extender (25 and 75 mmol) ($P<0.05$).

* **Corresponding Author:** Khadijeh Piri ✉ khadijehpiri@yahoo.com

Introduction

Frozen spermatozoa are use in large quantity for Artificial Insemination (AI) worldwide but freezing and freeze-thawing process causes to release free oxidative radicals in semen which have negative effects on sperm characteristics e.g. mortality, intercellular enzyme activity, productivity and so on (Chen *et al.,* 1993; Rasul *et al.,* 2003; Bansal *et al.,* 2011). One of the factors on sperm freezing is additives which use to dilute semen. Extenders are named for substances which can protect spermatozoa from temperature changes and provide nutritional needs of sperm while freezing (Ranjhan and Pathak, 1993; Patra *et al.,* 2001; Rasul *et al.,* 2003). It is reported that sugars can protect freeze spermatozoa by their interaction with membrane phospholipids. Raffinose is a high molecular three saccharide ($C_{18}H_{22}O_{16}.5H_2O$) which consist of Glucose, Fructose and Galactose (Bansal *et al.,* 2011).

Raffinose has less permeability through cell membrane and can impress its effects via membrane lipids and proteins of sperm. This interaction cause to diminish ice crystal formation in spermatozoa and inhibits osmotic pressure changes (Agca *et al.,* 2002; Bansal *et al.,* 2011).

Trehalose is a disaccharide ($C_{12}H_{22}O_{11}.2H_2O$) which contains two glucose units and open-chain form. Trehalose is found in large quantity in fang, yeast and some bacteria (Aboagla and Terada, 2003). Previous researches have shown Trehalose has ability to protect membrane protein deformation caused by temperature changes in spermatozoa (Hami and Molla Hoseyni, 2011).

Based on what mentioned above, researches, the main purpose of the current study was to investigate possible effects of different levels of Raffinose and Trehalose in Andromed semen diluter on post semen-thawing characteristics of buffalo spermatozoa.

Material and methods

Animals and semen collection

Semen samples from four male buffalo (4.2±0.04 year-old) were used in this study. All experiment procedure was done in Uremia Genetics and Buffalo Breeding Research Center, west Azarbayjan, Iran. Semen samples procedure done using the artificial vagina two times per week in spring. All ejaculations have done from 8 to 9 A.M. The ejaculates were evaluated for motility using microscopic investigation.

Semen processing

Semen was diluted using commercial Andromed contacting different levels of Raffinose (Sigma-Aldrich, 83400) (0, 25 and 75 Mmol) and Trehalose (Sigma-Aldrich, 0167) (0, 25 and 75 Mmol) at 37 °C. Semen was cooled to 4 °C for a period of 4 h and loaded into 0.05 mL straws contain 20×10^6 cells/mL using automatic packing system, frozen and stored in liquid nitrogen for 2 weeks. After that frozen semen pull out form the liquid nitrogen and forty second period used to freeze- thawing process at 37 °C. There were 5 replications for each diluted semen sample.

Semen evaluation

Spermatozoa motility, Progressive sperm (P), Progressive sperm high-speed (A), Progressive sperm slow-speed (B), Movement of sperm (C), Without moving (D), Curvilnear Velocity (VCL), Straight-line Velocity (VSL), Linear index (LIN), Average path Velocity (VAP), Amplitude of Lateral Head Displacement (ALH) and Beat–Cross Frequency (BCF) have determined using microscope and computer evaluation system at zero and 6 h post semen-thawing.

Statistical analysis

Data was analyzed and by Multivariate Analysis of Variance by the linear model using SAS statistical software (SAS 9.1, 2001). Comparative analysis of the mean of treatments was performed using Tukey's Studentized Range (HSD) test. $P<0.05$ was considered as significant differences between treatments.

Results

Effects of different levels of Raffinose (0, 25 and 75 Mmol) and Trehalose (0, 25 and 75 Mmol) on post semen-thawing characteristics of buffalo spermatozoa at zero and 6 h post semen-thawing are presented in table 1 to 4. According to the data, addition of different levels of Raffinose (0, 25 and 75 Mmol) and Trehalose (0, 25 and 75 Mmol) to the semen, significantly decreased motility, P, VCL, VSL, VAP, LIN, ALH and STR of spermatozoa ($P < 0.05$). Furthermore, there was no significant effect on Progressive sperm slow-speed (B) and Movement of sperm (C), ($P > 0.05$) (Table 1 and 2).

Table 1. Effect of different levels of Raffinose and Trehalose in Andromed semen diluter on post semen-thawing quality characteristics of buffalo spermatozoa.

Treatment combination			Spermatozoa characteristics							
diluted	Rafinose(Mm)	Trehalose(Mm)	motility	P (%)	Type A	Type B	Type C	Type D	VCL (Mm/s)	VSL (Mm/s)
Andromed	0	0	55^{a}	51.324^{a}	19^{a}	31.984^{a}	3.666^{a}	45^{b}	24.752^{a}	12.972^{a}
Andromed	0	25	33.56^{b}	30.968^{b}	14.05^{b}	16.92^{b}	2.592^{a}	66.44^{a}	19.45^{a}	9.334^{a}
Andromed	0	75	19.176^{c}	18.176^{c}	6.894^{c}	11.284^{b}	1^{b}	80.824^{a}	12.125^{b}	5.052^{b}
Andromed	25	0	21.686^{c}	19.746^{c}	3.294^{c}	16.45^{b}	1.94^{b}	78.314^{a}	13.466^{b}	4.13^{b}
Andromed	25	25	9.472^{d}	5.594^{d}	0	5.594^{c}	3.876^{a}	90.528^{a}	12.526^{b}	2.148^{b}
Andromed	25	75	15.592^{c}	12.416^{c}	3.693c	8.724^{c}	3.176^{a}	84.408^{a}	14.486^{b}	3.746^{b}
Andromed	75	0	12.674^{c}	11.08^{c}	1.95^{c}	9.132^{c}	1.594^{b}	87.326^{a}	13.272^{b}	2.848^{b}
Andromed	75	25	25.748^{c}	23.376^{b}	9.344^{b}	14.032^{b}	2.37^{a}	74.252^{a}	15.924^{b}	6.106^{b}
Andromed	75	75	3.912^{d}	2.94^{d}	0	2.94^{d}	$.976^{b}$	96.88^{a}	10.232^{c}	1.674^{b}
SEM			0.175	0.177	0.175	0.177	0.098	0.148	0.039	0.175
P Value			0.003	0.004	0.037	0.129	0.273	0.003	0.016	0.007

There are significant differences between groups with different codes in a column (superscript letters a, b; $p \leq 0.05$).

Table 2. Effect of different levels of Raffinose and Trehalose in Andromed semen diluter on post semen-thawing quality characteristics of buffalo spermatozoa.

Treatment combination			Spermatozoa characteristics				
diluted	Rafinose(Mm)	Trehalose(Mm)	VAP(Mm/s)	MAD(D)	ALH(Mm/s)	BCF(HZ)	LIN(%)
Andromed	0	0	15.766^{a}	28.896^{a}	1.2^{a}	3.796^{a}	43.538^{a}
Andromed	0	25	11.53^{a}	21.68^{a}	1.042^{a}	2.34^{a}	36.504^{b}
Andromed	0	75	7.096^{b}	11.506^{b}	0.808^{b}	1.412^{b}	23.728^{c}
Andromed	25	0	6.152^{b}	13.394^{b}	0.852^{b}	1.306^{b}	23.874^{c}
Andromed	25	25	4.46^{b}	14.762^{b}	0.858^{b}	0.99^{c}	16.512^{d}
Andromed	25	75	6.102^{b}	18.744^{b}	0.96^{b}	1.454^{b}	18.734^{d}
Andromed	75	0	5.09^{b}	8.282^{c}	0.862^{b}	1.49^{b}	16.696^{d}
Andromed	75	25	8.38^{b}	16.626^{b}	0.934^{b}	2.172^{a}	30.658^{b}
Andromed	75	75	3.404^{b}	10.522^{c}	0.706^{b}	0.576^{c}	13.83^{d}
SEM			0.047	0.11	0.002	0.013	0.185
P Value			0.004	0.06	0.033	0.063	0.036

There are significant differences between groups with different codes in a column (superscript letters a, b, c, d; $p \leq 0.05$).

In our study, a significant decrease on motility indexes in all treatment groups of spermatozoa at 6 h post semen-thawing (P <0.05) (table 3 and 4).

Table 3. Effect of different levels of Raffinose and Trehalose in Andromed semen diluter on post semen-thawing quality characteristics of buffalo spermatozoa.

Treatment combination			Spermatozoa characteristics							
diluted	Rafinose(Mm)	Trehalose(Mm)	motility	P (%)	Type A	Type B	Type C	Type D	VCL (Mm/s)	VSL (Mm/s)
AndroMed	0	0	11.732a	8.842a	2.096b	6.744a	2.888a	88.268a	13.716a	2.654b
AndroMed	0	25	14.576a	10.34a	6.332a	7.106a	1.236a	85.424b	13.76a	4.254a
AndroMed	0	75	9.904b	8.566a	3.276b	5.292a	1.338a	90.096a	12.2a	3.014a
AndroMed	25	0	4.786b	2.852b	0.452c	2.402b	1.932a	95.216a	11.496a	1.492b
AndroMed	25	25	0.634c	0.264c	0	0.264c	0.37b	99.366a	10.308b	0.964c
AndroMed	25	75	1.494c	0.964c	0	0.964c	0.53b	98.506a	10.466b	0.922c
AndroMed	75	0	1.362c	0.308c	0.154c	0.154c	1.054a	98.638a	10.466b	0.986c
AndroMed	75	25	0.57c	0.192c	0	0.192c	0.384b	99.424a	9.834b	1.354b
AndroMed	75	75	0.312d	0	0	0	0.312b	99.688a	9.544b	0.984c
SEM			0.046	0.049	0.026	0.038	0.028	0.046	0.024	0.14
P Value			0.001	0	0	0.079	0.869	0	0.005	0

There are significant differences between groups with different codes in a column (superscript letters a, b, c; p ≤ 0.05).

Table 4. Effect of different levels of Raffinose and Trehalose in Andromed semen diluter on post semen-thawing quality characteristics of buffalo spermatozoa.

Treatment combinatio			Spermatozoa characteristics				
diluted	Rafinose(Mm)	Trehalose(Mm)	VAP(Mm/s)	MAD(D)	ALH(Mm/s)	BCF(HZ)	LIN(%)
AndroMed	0	0	4.894a	17.784a	0.896a	1.502a	16.73a
AndroMed	0	25	6.298a	14.985a	0.828a	1.554a	36.504b
AndroMed	0	75	4.802a	13.01a	0.792b	1.318a	23.728c
AndroMed	25	0	3.528b	12.732b	0.81a	0.968b	23.874c
AndroMed	25	25	3.064b	10.764b	0.766b	0.868b	16.512d
AndroMed	25	75	2.808c	9.914b	0.748b	0.524b	18.734d
AndroMed	75	0	2.984c	10.598b	0.756b	0.814b	16.696d
AndroMed	75	25	3.014b	7.624c	0.728b	0.446b	30.658b
AndroMed	75	75	2.628c	9.388b	0.714b	0.558b	13.83d
SEM			0.015	0.075	0.001	0.006	0.113
P Value			0	0.005	0.079	0	0.22

There are significant differences between groups with different codes in a column (superscript letters a, b, c, d; p ≤ 0.05.

Discussion

According to the current results, administration of antioxidants such as Raffinose and Trehalose and the possible interaction of these antioxidants seems is the reason of lessening in spermatozoa quality characteristics at 6 h post freeze-thawing. In this regard, Livine *et al.,* (1999) and Pathra *et al.,* (2001) reported Raffinose impresses its suppressive effects by its interaction with membrane protein and lipids which leads to dehydration of sperm during freezing phase. Conversely, it is reported Raffinose protects spermatozoa by diminish ice crystal formation and osmotic pressure change (Storey *et al.,* 1998; Rasul *et al.,* 2003). Also, some reports claim Tris based Raffinose and Trehalose semen diluters improved quality of frozen spermatozoa in sheep (Fiser and Fairfull, 1989). Additionally, Trehalose diluters amplified antioxidant activity and decreased oxidative stress in freezing procedure in ram semen (Aisen *et al.,* 2005). In this study, administration of zero, 25 and 75 Mmol Raffinose and Trehalose to Andromed diluter decreased spermatozoa motility at 0 and 6 h post freeze-thawing in buffalo which is in disagreement with previous researches. It seems, different results can related to dosage of Raffinose and Trehalose, diluter composition, type of animal and freeze thawing period. In this regard, Hu *et al*., (Bucak and Tekin, 2007; Hu *et al.,* 2009) reported Trehalose diluter increase's Catalase, Glutathione peroxidase activity in buck semen. In agreement, it is suggested acid citric and Trehalose diluter based on Tris improved spermatozoa quality in freezing phase in bulk (Aboagla and Terada, 2003). In conclusion, addition of antioxidant substances such as Raffinose and Trehalose to Andromed commercial diluter not only fails to improve frozen spermatozoa indexes, but also decreased spermatozoa motility and quality parameters in buffalo semen. Also, we think further researches needs to elucidate the direct effects of Raffinose and Trehalose on Andromed base diluter in buffalo semen.

References

Aboagla EM, Terada T. 2003. Trehalose-enhanced fluidity of the goat sperm membrane and its protection during Freezing. Biology Reproduction, **69,** 1245-1250. http://dx.doi.org/10.1095/biolreprod.103.017889

Agca Y, Gilmore J, Byers M, Woods E, liu J, critser JK. 2002. Osmotic Characteristics of mouse spermatozoa in the presence of extender and sugers. Biology Reproduction **67,** 1493-1510. http://dx.doi.org/10.1095/biolreprod.102.005579

Aisen E, Quintana M, Medina V, Morello H, Venturino A. 2005. Ultramicroscopic and biochemical changes in ram spermatozoa cryopreserved with trehalose-based hypertonic extenders. Cryobiology **50,** 239-249. http://dx.doi.org/10.1016/j.cryobiol.2005.02.002

Bansal AK, Bilaspuri GS. 2011. Impacts of Oxidative Stress and Antioxidants on Semen Functions.Review Article Veterinary Medicine Inter **7,** 1-12. http://dx.doi.org/10.4061/2011/686137

Bucak MN, Tekin N. 2007. Protective effect of taurine, glutathione and trehalose on the liquid storage of rams semen. Small Ruminant Research **73,** 103-108. http://dx.doi.org/10.1016/j.smallrumres.2006.12.001

Chen Y, Foote RH, Brockett CC. 1993. Effect of sucrose, trehalose, hypotaurine, taurine and blood serum on survival of frozen bull sperm. Cryobiology **30,** 23-31. http://dx.doi.org/10.1006/cryo.1993.1042

Fiser PS, Fairfull RW. 1989. The effect motility and acrosomal integrity of ram spermatozoa. Cryobiology **26(1),** 64-69.

Hami K, Molla Hoseyni, M. 2011. Trehalose production by yeasts and biotechnological effects as feed additive. First National food safety PP 1. (In Persian).

Hu JH, Li QW, Li G, Jiang ZL, Bu SH, Yang H,

Wang LQ. 2009. The cryoprotective effect of trehalose supplementation on boar spermatozoa quality. Animal Reproduction Science **112,** 107-118. http://dx.doi.org/10.1016/j.anireprosci.2008.04.009

Livine RL, Berlett BS, Moskovitz J, Mosoni L, Stadtman ER. 1999. Methionine residues may protect proteins from critical oxidative damage, Mech. Ageing Devineu **107,** 323-332.

Patra RC, Swarup D, Dwivedi SK. 2001. Antioxidant effect of alphatocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicology **162,** 81-88.
http://dx.doi.org/10.1016/S0300-483X(01)00345-6

Ranjhan SK, Pathak NN. 1993. Text book on buffalo production, 3rd, Masjid Rod. Tangpura. NewDehli **20,** 25-25.

Rasul Z, Anzar M, Jalali S, Ahmad N. 2003. Effect of buffering system on post-thaw motion characteristics, plasma membrane integrity, and acrosome morphology of buffalo spermatozoa. Animal Reproduction Science **59,** 31-41.

SAS Institute. SAS state software: Changes and Enhancement through release 8.2. SAS institute, 2001; Inc; Cary, NC.

Storey BT, Noiles EE, Thompson KA. 1998. Comparison of glycerol, other polyols, trehalose and raffinose to provide a defined cryoprotectant medium for mouse sperm cryopreservation. Cryobiology **37,** 46-58.
http://dx.doi.org/10.1006/cryo.1998.2097