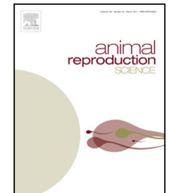
Animal Reproduction Science 167 (2016) 83–88

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci



Royal jelly supplementation in semen extender enhances post-thaw quality and fertility of Nili-Ravi buffalo bull sperm

Qaisar Shahzad[a,*], Muhammad Usman Mehmood[b], Hamayun Khan[c], Asma ul Husna[d], Saima Qadeer[d], Asima Azam[d], Zahid Naseer[e], Ejaz Ahmad[f], Muhammad Safdar[g], Mushtaq Ahmad[b]

[a] *Reproduction Division, Buffalo Research Institute Pattoki, Kasur 55050, Pakistan*
[b] *Department of Theriogenology, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan*
[c] *Department of Animal Health, University of Agriculture, Peshawar 25120, Pakistan*
[d] *Department of Zoology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46000, Pakistan*
[e] *Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Adnan Menderes University, Aydin 09016, Turkey*
[f] *Department of Clinical Sciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan 54000, Pakistan*
[g] *Department of Medical Biology and Genetics, Gaziantep University, Gaziantep 27310, Turkey*

ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form 2 February 2016
Accepted 9 February 2016
Available online 11 February 2016

Keywords:
Buffalo bull
Royal jelly
Cryopreservation
In vitro fertilization
Fertility

ABSTRACT

Two experiments were conducted to evaluate the effect of royal jelly (RJ) on post-thaw sperm quality, *in vitro* and *in vivo* fertility rate of cryopreserved buffalo bull sperm. The semen was collected from three mature regular donor buffalo bulls, ejaculates were pooled and semen evaluated initially. In Experiment 1, the ejaculates were extended in tris-citric acid diluter supplemented with different RJ concentrations (0, 0.05, 0.1, 0.2, 0.3 or 0.4%). The diluted semen was cooled to 4 °C, packaged into 0.5 mL straws and frozen using standard procedure. The straws were thawed and assessed for sperm progressive motility, viability, plasma membrane, acrosome, and chromatin integrity. The results indicated that sperm progressive motility was significantly greater ($P<0.05$) in 0.05, 0.1, 0.2 and 0.3% RJ than 0.4% RJ supplemented and control groups. The sperm viability, plasma membrane and acrosome integrity were significantly improved ($P<0.05$) in 0.1% RJ supplemented group the compared to other treatment groups.

In Experiment 2, cryopreserved sperm with 0.1% RJ supplementation and control (without RJ supplementation) were used to observe the *in vitro* fertilizing potential and *in vivo* fertility. *In vitro* fertilization method was applied to assess the cleavage rate; whereas, AI was performed in buffalo during *in vivo* fertility trial. The buffaloes were inseminated 12 h after standing estrus and pregnancy diagnosis was performed through ultrasonography. The results revealed that the cleavage rate was higher ($P<0.05$) in 0.1% RJ as compared to control group. However, the pregnancy rate was similar ($P>0.05$) between 0.1% RJ supplemented and control groups. It is concluded that supplementation of RJ in freezing extender can improve the cryosurvival rate and *in vitro* fertilizing capacity of buffalo bull sperm.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The buffalo (*Bubalis bubalis*) production has been benefited from the progress in sperm cryopreservation and

* Corresponding author.
E-mail address: raoqaisarshahzad@gmail.com (Q. Shahzad).

http://dx.doi.org/10.1016/j.anireprosci.2016.02.010
0378-4320/© 2016 Elsevier B.V. All rights reserved.

Table 1
Effect of royal jelly on post-thaw progressive motility (%), plasma membrane integrity (%), acrosomal integrity (%) and chromatin integrity (%) of cryopreserved buffalo bull sperm.

Parameters	RJ concentrations (%)					
	0	0.05	0.1	0.2	0.3	0.4
Sperm progressive motility (%)	38.3 ± 4.3^{c}	53.3 ± 14.7^{ab}	59.1 ± 9.1^{a}	53.3 ± 2.1^{ab}	51.6 ± 3.7^{ab}	44.2 ± 3.5^{bc}
Sperm viability (%)	47.6 ± 1.5^{c}	48.5 ± 6.1^{c}	67.0 ± 3.8^{a}	51.4 ± 5.8^{b}	51.0 ± 1.2^{b}	49.0 ± 1.7^{bc}
Sperm plasma membrane integrity (%)	47.0 ± 3.2^{d}	55.3 ± 12.1^{bc}	62.3 ± 2.5^{a}	60.2 ± 3.4^{ab}	60.0 ± 1.5^{abc}	54.0 ± 6.8^{cd}
Sperm acrosome integrity (%)	53.5 ± 2.2^{d}	68.3 ± 14.0^{b}	75.0 ± 6.4^{a}	64.8 ± 3.1^{bc}	61.2 ± 2.5^{c}	60.0 ± 5.1^{c}
Sperm chromatin integrity (%)	97.8 ± 1.3	98.0 ± 1.1	98.6 ± 0.5	98.5 ± 1.2	98.1 ± 0.3	97.8 ± 0.4

Data is presented in Mean ± S.D.
Different superscripts along the rows indicate the significant differences ($P < 0.05$) among groups.

artificial insemination (AI) technology. Since four to five decades, the buffaloes are being inseminated artificially by using cryopreserved semen, however; the conception rate is less comparatively to the cattle (Barile, 2012; Anzar et al., 2003). There are several intrinsic and extrinsic attributes that reduce fertility in buffalo; however, damage to sperm during cryogenic procedures is the major impediment for AI application in buffalo (Watson, 1995).

The freeze-thaw process leads to structural and functional damages by excessive generation of reactive oxygen species (ROS) (Guthrie and Welch, 2006). In addition, predominance of polyunsaturated fatty acids in sperm plasma membrane instigates the lipid peroxidation process which in turn reduces the motility, viability, DNA integrity and consequently pregnancy rate (Nair et al., 2006; Andrabi, 2009). Cryopreservation phases such as dilution, cooling, equilibration and freezing weaken the naturally existing seminal antioxidants capacity. In this context, the exogenous antioxidants supplementation in semen extender has provided a great opportunity to improve sperm quality to combat the oxidative stress exerted by cryopreservation (Bansal and Bilaspuri, 2011).

RJ (bee's milk) is secreted by the hypopharyngeal glands of worker bees primarily for feeding larvae and maintaining the queen bee (Townsend and Lucas, 1940). It contains proteins, lipids, sugars, vitamins (A, B5, C, D, E) and essential amino acids particularly cystine, lysine and arginine (Howe, 1985; Kodai et al., 2007). Perusal to literature, the RJ has been used as pharmacologic agent to manage different diseased conditions (Fujii et al., 1990; Kamakura et al., 2001; Matsui et al., 2002). It has also been documented that the amino acid present in RJ have high antioxidant potency and scavenging ability against free radicals such as superoxide anion radical, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, and hydroxyl radical (Robak and Marcinkiewicz, 1994; Karadeniz et al., 2011). Moreover, the beneficial role of RJ supplementation on male fertility has reported in lab model animals (Elnagar, 2010; Zahmatkesh et al., 2014; Ghanbari et al., 2015). Similarly, a few studies have been conducted in order to determine the effect of RJ on sperm quality during incubation at 37 °C or cold storage in domestic animals (Abd-Allah, 2012; Moradi et al., 2013). However, the putative effect of RJ supplementation on mammalian sperm cryosurvival has not been tested yet. Therefore, the present study was designed to determine the effect of RJ supplementation in extender on motility, viability, plasma membrane, acrosomal, and chromatin integrity, and fertility (*in vivo* and *in vitro*) of cryopreserved buffalo sperm.

2. Materials and methods

2.1. Experiment 1. Effect of different RJ concentrations on cryosurvival rate of buffalo bull sperm

2.1.1. Animals and semen collection

The present study was conducted at Semen Production Unit, Qadirabad, Sahiwal (30°43′0″N, 73°57′0″E) and Buffalo Research Institute, Pattoki, Kasur (31°1′0″N, 73°51′0″E), Punjab, Pakistan during peak breeding season (September–November 2013). Semen was collected from regular semen donor Nili Ravi buffalo bulls (n = 03; 4–6 years age) using pre-warmed artificial vagina. The both ejaculates of each bull were pooled if contained minimum attributes (>0.5 mL volume, >0.5 × 10^9 sperm mL concentration, >70% motility and <20% morphological abnormal) and used for further processing. However, the samples of individual bulls were processed separately. A total of nine replications were performed using eighteen ejaculates from three bulls.

2.1.2. Semen processing

The basic extender was tris-citrate egg yolk extender contained [247.6 mM tris-(hydroxymethyl)-aminomethane (Research Organics, Cleveland, OH, USA), 74.2 mM citric acid (Fisher Scientific, Loughborough, Leicestershire, UK), 0.2% w/v fructose (Scharlau, Barcelona, Spain)] 20% (v/v) egg yolk, 7% (v/v) glycerol (Merck, Darmstadt, Germany), benzyl penicillin (1000 IU mL^{-1} and streptomycin sulphate (1000 μg mL^{-1}). The samples were first extended with basic extender only and sperm concentration was fixed in each replicate. In the second step RJ supplemented extender (0, 0.05, 0.1, 0.2, 0.3 or 0.4%) was added and final sperm concentration 50 × 10^6/mL was achieved. Diluted semen was gradually cooled to 4 °C in 2 h and equilibrated for further 2 h at 4 °C. After equilibration, cooled semen was packaged in 0.5 mL French straws and placed horizontally over liquid nitrogen vapors (5 cm above the liquid nitrogen level) for 10 min. Straws were then plunged and stored into liquid nitrogen (−196 °C). After 24 h of storage in liquid nitrogen, three semen straws from each treatment group in each replicate were thawed at 37 °C for 30 s and analyzed for the following parameters.

Download English Version:

https://daneshyari.com/en/article/2072612

Download Persian Version:

https://daneshyari.com/article/2072612

Daneshyari.com