Polish Journal of Veterinary Sciences Vol. 22, No. 1 (2019), 177–179

DOI 10.24425/pjvs.2019.127084

Original article

Quality parameters and fertility of ram semen cryopreserved in egg yolk and soybean lecithin supplemented extenders

P. Gogol, M. Bryła, M. Trzcińska, M. Bochenek

Department of Animal Reproduction Biotechnology,
National Research Institute of Animal Production , Kraków , 32-083 Balice/Kraków, Poland

Abstract

The aim of the study was to investigate the effect of soybean lecithin as a substitute for egg yolk in milk and tris based extenders in ram semen cryopreservation. Twenty ejaculates were collected from four healthy, mature Wrzosówka rams (2-3 years of age). Each ejaculate was divided into four equal aliquots and diluted with four different extenders: 1) milk extender containing 5% egg yolk, 2) milk extender containing 1.5% soybean lecithin, 3) tris extender containing 20% egg yolk, 4) tris extender containing 1.5% soybean lecithin. Extended semen was loaded into 0.25 ml French straws, cooled and frozen in liquid nitrogen vapor. Total motility, curvilinear velocity, plasma membrane integrity and fertilizing ability of sperm were assessed after thawing. Total motility was lower ($p<0.05$) in tris-soybean lecithin extender when compared to other extenders. Curvilinear velocity was higher ($p<0.05$) for spermatozoa cryopreserved in milk-soybean lecithin extender compared to other extenders tested. For the percentage of live sperm no significant difference was observed between extenders. The lambing rate were higher (not statistically significant) in ewes inseminated with semen doses frozen in milk-soybean lecithin extender (42.9%) than in the tris-egg yolk extender (16.7%). In conclusion, replacing the egg yolk with soybean lecithin was effective in milk but not in tris extender.

Key words: ram semen, cryopreservation, soybean lecithin, sperm quality, fertility

Introduction

Soybean lecithin is considered to be a chemically-defined pathogen-free alternative to egg yolk in semen extenders for cryopreservation of ram spermatozoa. It protects the sperm membrane by stabilizing and replacing phospholipids, thus increasing tolerance to the freezing process. During the cryopreservation of ram semen, tris extender supplemented with 1.5% soy lecithin enhance most semen quality parameters (Forouzanfar et al. 2010, Emamverdi et al. 2013). Nevertheless, the negative effect of soybean lecithin on sperm motility and mitochondrial activity has also been observed in frozen-thawed ram semen (Del Valle et al. 2012, Mata-Campuzano et al. 2015). To our knowledge, the effect of replacing egg yolk with soybean lecithin in a milk extender has not been studied so far. The aim of this study was to investigate the effect

Correspondence to: P. Gogol, e-mail: piotr.gogol@izoo.krakow.pl, tel.: +48 666081282

Table 1. Motility parameters, membrane integrity and fertility of frozen-thawed ram semen in the tested extenders.(Mean±SD).

Extender	Total motility (%)	VCL (μm/s)	Live cells (%)	Lambing rate (%)
Milk-egg yolk	52.4±14.1a	75.0±19.3a	23.9±9.3a	-
Milk-soybean lecithin	59.4±15.1a	95.8±18.6b	27.3±12.6a	42.9 (6/14)a
Tris-egg yolk	55.9±15.2a	78.6±15.5a	28.6±11.8a	16.7 (2/12)a
Tris-soybean lecithin	41.9±13.8b	77.6±12.8a	27.2±10.0a	-

Values in columns with different letters (a,b) are significantly different ($p<0.05$)
VCL – curvilinear velocity

of soybean lecithin as a substitute for egg yolk in milk and tris extender in ram semen cryopreservation.

Materials and Methods

Semen was collected from four healthy, mature Wrzosówka rams (2-3 years of age). A total of 20 ejaculates (five ejaculates for each ram) were collected twice a week from the rams using an artificial vagina, during the breeding season (autumn). After collection, each ejaculate was divided into four equal aliquots and diluted with four different extenders: 1) milk extender containing 5% egg yolk, 2) milk extender containing 1.5% soybean lecithin, 3) tris extender containing 20% egg yolk, 4) tris extender containing 1.5% soybean lecithin. A milk extender (reconstituted 11% skim milk powder, 1g/100 ml fructose, 5% glicerol) and tris extender (300 mM Tris, 28 mM glucose, 95 mM citric acid, 5% glicerol) were used. Extended semen was loaded into 0.25 ml French straws ($150x10^6$ sperm per straw) and held for 2 h at 4°C. The straws were then frozen in nitrogen vapor for 10 min and stored in liquid nitrogen until thawed and used for evaluation of sperm parameters and *in vivo* fertilization. Sperm motility parameters were assessed using a computer-assisted sperm analysis system (Sperm Class Analyzer, S.C.A V5.1, Microptic, Barcelona, Spain). A semen sample was placed in a Leja count 4 chamber slide (Leja Products B.V., The Netherlands) and a minimum of 500 sperm in 5 fields were analysed at 37°C using standard settings. The percentage of total motile spermatozoa and curvilinear velocity were determined. The sperm membrane integrity was assessed using a LIVE/DEAD® Sperm Viability Kit (SYBR-14 and propidium iodide (PI) (Molecular Probes, Inc., Eugene, Oregon, USA). Fluorescence in green and red bands was measured using a CytoFlex (BeckmanCoulter, USA) flow cytometer. The percentage of live cells with only a green fluorescent signal (SYBR-14 positive), moribund cells exhibiting a mixed green (SYBR-14 positive) and red (PI positive) fluorescence, and dead cells with only a red fluorescent signal (PI positive) were recorded. Tweny-six Wrzosówka ewes with 45-50 kg weight and 2-3 years old were used to determine the effect of the extender (semen was used frozen in two extenders ensuring the highest sperm quality after thawing: milk-soybean lecithin and tris-egg yolk) on fertilizing ability of sperm. All ewes were submitted to estrus synchronization using the standard protocol of 20 mg fluorogestone acetate-impregnated sponges (12 days of intravaginal treatment; Chronogest CR 20 mg, Intervet, Boxmeer, Holland) and eCG (500 IU im treatment at sponge withdrawal; Intergonan; Intervet). Ewes were intracervically inseminated ($150x10^6$ sperm per ewe) with frozen-thawed semen 60 hours after sponge withdrawal. After parturition the lambing rate was calculated.

Means were compared using the t-test and differences were considered significant at $p<0.05$. A Chi-square test was carried out to analyse the effect of the extender on the lambing rate.

Results and Discussion

Total motility was lower ($p<0.05$) in Tris-soybean lecithin extender when compared to other extenders (Table 1). Curvilinear velocity was higher ($p<0.05$) for spermatozoa cryopreserved in milk-soybean lecithin extender compared to other extenders tested. For the percentage of live sperm no significant difference was observed between extenders. The lambing rate were higher (not statistically significant) in ewes inseminated with semen doses frozen in the milk-soybean lecithin extender (42.9%) than in the tris-egg yolk extender (16.7%). The results of this study confirm that soybean lecithin can be successfully applied as a cryoprotectant for cryopreservation of ram semen (Forouzanfar et al. 2010, Emamverdi et al. 2013, Masoudi et al. 2016) but we concluded that replacing the egg yolk with soybean lecithin was effective in milk but not in tris extender.

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