Scientific Papers, Animal Science, Series D, vol. LV
CD-ROM ISSN 2285-5769, ISSN-L 2285-5750

EFFECT OF LOW DENSITY LIPOPROTEINS IN EXTENDER ON FREEZABILITY AND FERTILITY OF EGYPTIAN BUFFALO BULL SEMEN

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Abstract

Semen from three Egyptian buffalo bulls was collected once weekly and ejaculates with more 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 bulls effect. Seven extenders were used: Tris 20 % egg yolk extender with 7 ml glycerol as a control (T1), and substitution of whole egg yolk with 4, 6, 8, 10, 12 and 15 % low density lipoprotein (LDL), T2 – T6, respectively. Semen was diluted to 80 x10^6 sperm/ml, packaged into 0.25 ml straws, cooled, held at 5°C for 4 h, and then frozen in liquid nitrogen (LN) and stored at -196◦C for at least one month. Sperm progressive motility, intact acrosome and plasma membrane integrity were assesd at post dilution, equilibration, post-thawing (at 37°C for 30 sec.) and after 30 days storage in LN. This study reveled that LDL extenders were more effective in preservation of progressive motility, intact acrosome and integrity of the plasma membrane of buffalo spermatozoa than whole egg yolk extender. Sperm progressive, intact acrosome and plasma membrane integrity were much higher ($P < 0.05$) in the 12% LDL extender (63.3, 77.17 and 71.3% respectively) vs. 35, 40.8 and 34.7% in the control 20% EY extender at post-thawing process, respectively. Fertility rates were higher in extender containing 12% LDLs compared with the control (72.7% vs. 50%, respectively). It was concluded that LDL (12%) in extender improved the freezability and fertility of buffalo bull spermatozoa.

Key words: Buffalo Bull Semen; LDL; Freezability; Fertility

INTRODUCTION

Egg yolk (EY) is a common component of semen freezing extenders for most of the livestock species, including the buffalo (***Bubalus bubalis***) [1,2]. The major role of EY is to prevent sperm cell damage during the cooling and freezing processes. Egg yolk is generally used at a concentration of 20% (Vol./Vol.) in semen extender for bovine [3]. The use of EY in higher concentration may have deleterious effects combined with toxicity (amino acid oxidase activity) of dead spermatozoa resulting in lower post-thaw spermatozoal quality.

Andrabi et al. (2008) [4] reported that duck egg yolk compared to other avian yolks in extender improved the freezability of buffalo bull spermatozoa as judged by motility, survivability, and plasma membrane integrity, intactness of acrosome and head, mid-piece and tail abnormalities. They added that, the improvement or the decline in post-thaw quality of mammalian spermatozoa with EY is attributed to the differences in biochemical composition of the extender. Low density lipoproteins (LDL) contained in EY is largely responsible for sperm protection during cryopreservation [5,6]. The exact mechanism by which EY preserves the bull spermatozoa during freeze-thaw process is unknown [2].

Several studies have reported successful results with the addition and replacement of egg yolk by LDL in the semen freezing process of different species, like bull [7,8,9] ram [10] dogs [11] and buffalo bull semen

[12]. Therefore, the goals of this study were first to asses the cryoprotective effect of LDL as a replacement for chicken egg yolk, in extenders for Egyptian buffalo semen, on variables of semen quality after different stages of cryopreservation (i.e., after dilution, equilibration at 5° C and freezing-thawing and post-one month from freezing storage period in liquid nitrogen).

MATERIAL AND METHOD

Semen collection

Semen was obtained from three buffalo bulls at the International Livestock Management Training Center (ILMTC), Sakha Station belonging to the Animal Research Institute, Ministry of Agriculture, Egypt. Ejaculates with >75 % progressive motility and >85% normal sperm morphology prior to cryopreservation were used. One ejaculate was obtained from each bull using an artificial vagina for a period of 4 weeks.

Extender preparation

The extender for the treatment groups used in this study was composed as follow: 3.025g Tris, 1.675g citric acid, 0.75g glucose, 7 ml glycerol, 0.25 lincomycin, 0.005g streptomycin and different concentrations of LDL (4, 6, 8, 10, 12 and 15%) for 100 ml bi-distilled water. The extender for the control group differed from the treatment groups by replacing LDL with 20% egg yolk.

LDL extraction

The LDL was extracted from egg yolk according to the method described by [7] .

Semen processing

After the evaluation of motility and morphology, the fresh semen of three buffalo bulls was pooled and then divided into seven equal fraction, one fraction was diluted with the extender for the control group and others were diluted with the extender for the treatments to obtain 80 x 10^6 sperm/ml. Semen was cooled from 37 to 5ºC for 1.5h and maintained in a refrigerator at 5ºC for 4h. French straws (0.25 ml) were filled using a semen filling machine. Subsequently, these straws were placed 4 cm above the liquid nitrogen surface where the temperature was approximately -120ºC. After 10 min all straws were immersed directly into liquid nitrogen (-196º) for storage.

Semen quality assays

Assays for buffalo sperm motility, plasma membrane integrity and acrosome integrity were performed at post-dilution, equilibration, post-thaw and after 30 days of storage in liquid nitrogen. For thawing, straws were dipped into a water bath at 37ºC for 30 sec. Progressive motility was estimated according to [13], acrosome and membrane integrity were estimated according to [14, 15], respectively.

Fertility trial

A total of 141 Egyptian buffalo cows were artificially inseminated with random frozen doses from various extenders. Each female was inseminated with a single straw 8-14 h after start of estrous behavior. Using recto-vaginal technique and the universal insemination gun, the thawed semen was deposited in the uterine body just next to the anterior end of the cervix. Conception rate was confirmed by rectal palpation at least 60 days after insemination.

Statistical analysis

The data was statistically analyzed in two ways using general linear models procedure adapted by [16] for user's guide with one-way ANOVA. Duncan test within program SPSS was done to determine the differences among the means.

RESULTS AND DISSCUSION

Progressive sperm motility

Progressive motility (%) of buffalo bull spermatozoa at different stages of cryopreservation (post-dilution, post-equilibration and post-thaw) was superior ($P<0.05$) in the media containing LDL in comparison with control medium containing 20% EY (Fig.1). At post-dilution and

equilibration concentration of 12% LDL seemed to give the best results. Moreover, at post-thawing or at post 30 d storage in liquid nitrogen, sperm progressive motility was more twofold higher in the 12% LDL extender 63.3 or 61.7% vs. 35 or 26.7% in the control 20% EY extender (Fig.1).

The present results show clearly that, LDL can replace egg yolk in tris extender with better results in terms of progressive motility of buffalo spermatozoa. Furthermore, the optimum concentration of LDL has been determined to be 12%. In the bull, a concentration of 8% LDL gives the best results [7,8]. However, [12] demonstrated that the percentage of motile spermatozoa following different stages of cryopreservation in 10% LDL give the best results in Nili-Ravi buffalo bulls.

We suggested that the use of LDL could enhance the ability of buffalo spermatozoa against cold shock and improve the sperm quality during the freeze- thaw process in comparison with the medium based on egg yolk. Egg yolk is reported to contain some deleterious components which are potent to reduce semen motility[5]. The control medium is composed of 20% EY, which itself contains 50% dry matter, 6.6% of which is LDL [7,17].

The concentration of LDL in the control medium is therefore 6.6% LDL, which is very close to that use in the present 6-8% LDL medium (Fig.1), which gave the higher ($P < 0.05$) post- thaw progressive motility (45% and 50% for 6 and 8% LDL, respectively Fig.1) versus 35% in the control 20% EY. It is known that some components in egg yolk play an antagonistic role to the cryoprotective effect of LDL. This postulate may explain the higher post-thawed motility in extender containing 12% LDLs compared with egg yolk containing extender.

Acrosome and plasma membrane integrities

The extenders containing LDLs provided better protection for the acrosome than the medium containing 20% egg yolk ($P<0.05$) (Fig.2) following different stages of cryopreservation. The extender containing 12% LDL resulted in greater protection for the acrosome with an improvement of about 12.2%, 14.8%, 26.3% and 42.8% points over the control EY extender following dilution, equilibration, post-thaw and after 30 days storage in liquid nitrogen, respectively, (Fig.2).

The proportion of spermatozoa with an intact plasma membrane is superior in the medium containing 12% LDL than in the medium with egg yolk after dilution, equilibration, thawing and 30d storage in liquid nitrogen (87.7, 84.7, 71.3 and 68.5% vs. 75.5, 69.5, 34.7 and 25.8%, respectti-vely) (Fig. 3).

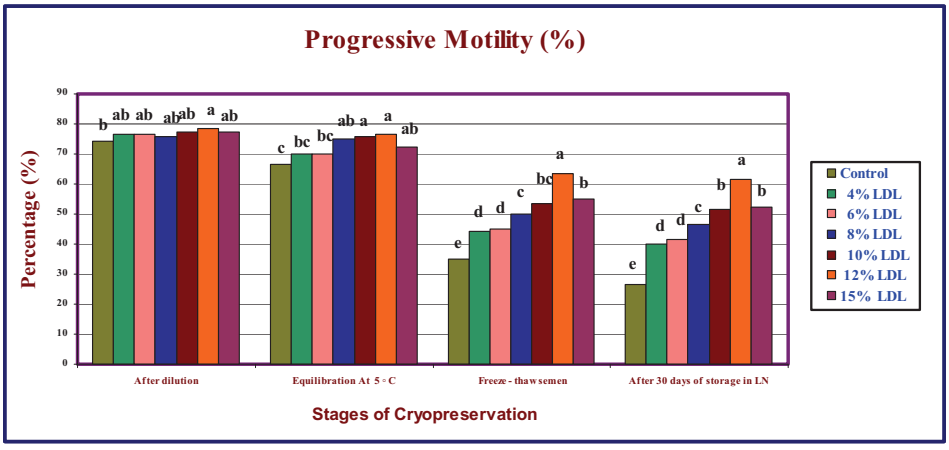

Fig. 1. Progressive motility of buffalo spermatozoa, at different stages of cryopreservation in different concentrations of LDL

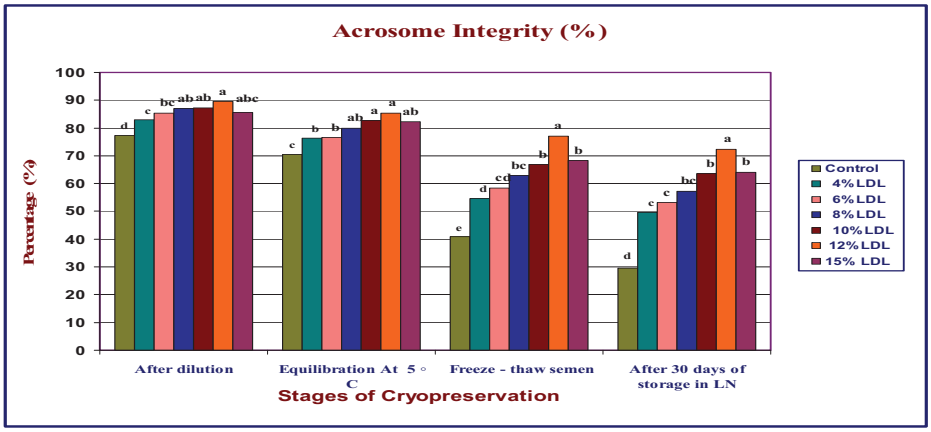

Fig. 2. Acrosome integrity of buffalo spermatozoa, at different stages of cryopreservation in different concentrations of LDL

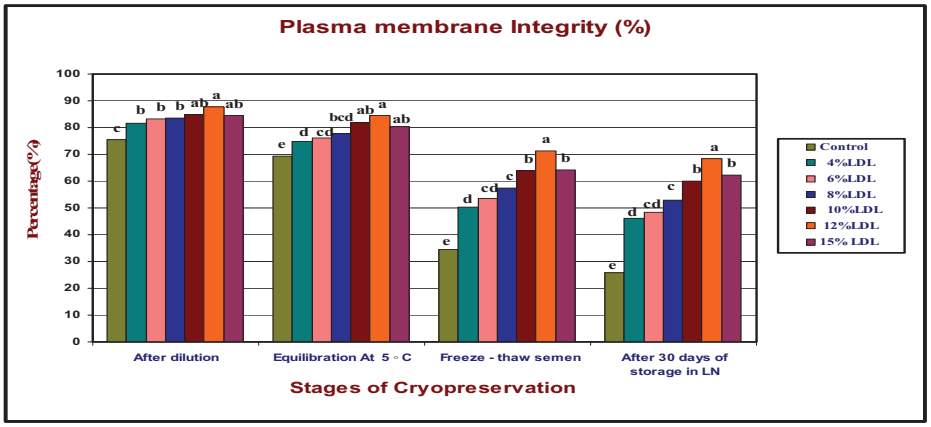

Fig. 3. Plasma membrane integrity of buffalo spermatozoa, at different stages of cryopreservation in different concentrations of LDL

The present results show that, the post-thaw structural and functional integrity of acrosome and plasma membrane of buffalo bull spermatozoa was higher in extender containing LDLs 12% compared with the control. **Amirat et al. (2004)** also reported in bulls that LDL did not induce more plasma membrane damage during the cryopreservation procedure than egg yolk. **Bencharif et al. (2008)** reported for dogs a better preservation of flagellar plasma membrane integrity for spermatozoa cryopreserved in LDL compared to egg yolk. In **1989, Courtens et al** [18] also reported that LDL were less aggressive to cell than egg yolk; there was alteration of the plasma membranes and very little acrosome disruption. They emphasized the possible adverse effect of calcium, present in high concentration in egg yolk. According to these authors, the acrosomes were modified or damaged, which could result from a rapid calcium influx into spermatozoa when the temperature is below 30ºC. Besides anthor interesting aspect that could be evaluated in impact of oxidation on membrane stability[19].

The medium containing 12% LDL provides the best protection of acrosome integrity, possibly via a direct action through the exchange or repair of acrosomal membrane phospholipids or possibly simply because

the medium is less rich in progesterone than egg yolk due to the filtering effect of the dialysis membrane [17]. The progesterone found in egg yolk plays a role in the capacitation of spermatozoa in cattle [20], horses [21] and man [22], it appears to act via an extragenomic action on human spermatozoa, via the secondary activation of calcium channels leading to an increase in intracellular Ca^{++}, which may be responsible for the capacitation of spermatozoa.

Effect of LDLs in extender on fertility rates

Fertility rates (%) in buffalo inseminated with semen cryopreserved in extenders containing different LDLs concentration (4, 6, 8, 10, 12 and 15%) were higher ($P < 0.05$) than control EY (Table 1).

The final aim of buffalo bull sperm cryopreservation is the production of fertilized eggs after artificial insemination (AI). A successful AI requires that a significant number of viable, fertile sperm are delivered at the site of fertilization in appropriate time. Fertility rates were higher with semen cryopreserved in extender containing 12% LDLs compared with the control (72.7% vs. 50%, respectively).

Table 1. Conception rate of buffalo-cows inseminated with frozen semen cryopreserved in different concentrations of LDL

Item	No. of inseminated females	No. of conceived females	Conception rate (%)
Control	20	10	50.0
4% LDL	25	14	56.0
6% LDL	25	15	60.0
8% LDL	10	6	60.0
10% LDL	25	16	64.0
12% LDL	11	8	72.7
15% LDL	25	16	64.0
Overall of LDL treatment	121	75	61.9

In the bull, [8] obtained in vitro fertilization test higher cleavage rate with semen frozen in LDL than with Optidyl$^{(R)}$, a commercial egg yolk extender, but no difference was observed on the blastoeyst rate between the two extenders. In dogs, [17] showed that the presence of LDL in the freezing extender can preserve fertility potential of spermatozoa: 6 bitches were confirmed pregnant of 6 that were inseminated.

In the Nili-Ravi buffalo bulls, [12] obtained in vivo fertilization study, higher ($P < 0.01$) fertility rates with semen cryopreserved in extender containing 10% LDLs compared with the control whole EY (20%). Also, El-Sharawy, et al (2012) [23] showed that the addition of 10, 20 and 30 mM of glutamine to the 12 % LDL extender lead to an improvement of quality of buffalo frozen semen and offer higher conception rates (72.2, 73.3 and 66.6%, respectively).

In another study high conception rate was recorded with the use frozen-thawed semen containing 15% DEY, being 65.8% followed by 20% DEY (59.3%), 20% CHEY (58.6%) and 10% DEY (58.1%), but the differences were not significant [24]. It showed be mentioned that conception rate (64 -72%, Table 1) achived in the current study is satisfactory as compared the previous studies using various freezing and thawing techniques.

CONCLUSIONS

Based on the results of this study, it could be concluded that LDL possesses remarkable cryoprotective properties for freezing-thawing buffalo spermatozoa. Higher progressive motility percentage, acrosome and plasma membrane integrity and conception rates were achived with the use of 12% LDL as compared to 20 % egg yolk semen extender.Thus it was cocluded that the optimum concentration of LDL in this buffalo semen freezing extender was 12%.

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