European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 11, 2020

THE EFFECT OF LYOPHILIZED LOW-DENSITY LIPOPROTEINS, VITAMIN E, AND THEIR MIXTURE TO TRIS DILUTE IN HOLSTEIN BULLS SEMEN QUALITY

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Abstract:
This experiment was conducted to determine the effect of lyophilized Low Density Lipoproteins (LDL) at 5%, Vitamin E at 0.8% and their mixture in Tris diluent, and its effect on the percentage of individual movement and live sperm and the percentage of total deformities after preservation by cooling and cryopreservation for (48 hours, 1 month and 2 months) in the semen of Holstein bulls, from September 2020 to January 2021. It was in two stages, the first phase of extraction and freezing, from September to October, LDL was extracted from egg yolk and converted into powder and kept in tight cans, as for the second stage, it was in the examination laboratory of the Veterinary Hospital, Al-Muthanna Governorate.Semen was collected from two Holstein bulls, with ages from 2-2.5 years, using an artificial vagina.The sample was divided and distributed to the experimental parameters T2, T3 and T4 using lyophilized LDL and vitamin E and their mixture in semen diluent and compared with the control group (20% egg yolk).The results of the study indicated the superiority of treatment T4 (lyophilized LDL + vitamin E) in the percentage of individual motility and live sperm, a decrease in the percentage of total deformations after preservation by cooling 5 ° C and cryopreservation -196 ° C. Concluded from the foregoing the possibility of using T4 in semen diluent because of its ability to preserve sperm, improve the individual motility rate, and obtain the lowest percentage of deformities during the preservation period.

Keywords:Lyophilized low-density lipoproteins, vitamin E, Tris dilute, Holstein bulls semen quality.

Introduction

The process of preserving sperm by cryopreservation, it is considered the most efficient tool at present for maintaining sperm integrity, to prolong its storage life and to maintain the physiological value, to be used for the purpose of artificial insemination (Lemma, 2011; Crespilho*et al*., 2014).As artificial insemination is one of the most widespread technologies in the world, which farmed animal legacies were widely disseminated (Oliveira *et al*., 2013).To exceed the success rate of artificial

insemination, take attention to the quality of the semen, of preparing the diluent components prepared for and added to it (EL-Harairy*et al*., 2016; Eidan, 2016; Adewoying*et al*., 2017).The process of fat oxidation that accompanies the process of preserving semen by freezing, the dissolution of the sperm membranes leads to a decrease in the physiological processes of the cell, an increase in free radicals, they were kinds of oxygenated items, it was breaks down the essential fatty acids and breaks down a number of vitamins K, D, E, and A, and DNA was damaged, resulted in a lack of sperm movement and a failure in IVF, a deterioration in the percentage (Hamilton *et a*l., 2016; Crespilho*et al*., 2014).

The reason for the increase in the oxidative stress process, which is caused by the freezing of the semen, arises from the increase in the cellular oxidation process in the sperm, caused a decrease in the levels of both enzymatic and non-enzymatic defense particles (Sikka, 2004).Fat-soluble antioxidants must be used (Beconi*et al*., 1991).Such as vitamin E, which was one of the most important fat-soluble non-enzymatic antioxidants, has the ability to protect fatty acids and cholesterol, during an effective role in suppressing free radicals that work on fat oxidation (Burton *et al*., 1982; Navarro, 1998).Studies have indicated that the low-density lipoproteins, LDL, found in egg yolks, which were used in semen dilute, considered the basis for protecting the plasma membrane of the sperm cell (Moussa*et al*., 2002).
Therefore, this study came for the possibility of using lyophilized LDL and vitamin E and their mixture to dilute Tris, as an antioxidant and for its positive role in improving the cryopreservation sperm characteristics of Holstein bulls, and to reduce damage to the plasma membrane and the integrity of the chromosome, as well as working to protect the genetic material and thus increase the fertility of cows.

Materials and Methods:

This study was carried out in the examination laboratory of the Veterinary Hospital, Al-Muthanna Governorate, from September 2020 to January 2021. 2 bulls were used, trained to collect semen using an artificial vagina, 2-2.5 years age, 450-500 kg body weight. The animals were housed in semi-open pens, collecting 1ml per ejaculate to collect semen to eliminate individual bulls.Bulls were subjected to the (FMD), (HS) and (Black Leg) vaccination programs during the research period.
A basal feeding system was used, as concentrated feed was provided daily at a rate of 4-6 kg/ animal, feed ingredients provided: 35% barley, 33% wheat bran, 10% yellow corn, 20% soybean meal, 1% vitamins and minerals, 0.5% table salt, 0.5% limestone. The crude protein reached 18% in the diet, and the amount of energy was 3323 kilocalories / kg.The coarse feed ranged between 7-9 kg / animal / day, green diet 50-60 kg / day, with the availability of drinking water.
Low-density lipoproteins were extracted from chicken egg yolks, according to a series of steps according to the method (Moussa, 2002), then put it in airtight and sterilized cans until it is lyophilize, each semen sample was diluted, add vitamin (E) and (LDL), dried individually, and their mixture to the Tris diluent in the proportions of

lyophilized LDL (5%), and vitamin E by (0.8%) m/ mol. Its effect was studied at 5 ° C, 48 hours, one and two months of cryopreservation.

A Factorial Experiment with Completely Randomized Design (CRD) were used, to study the effect of treatment and evaluation periods on experience characteristics, the statistical program (SAS, 2010) was used, by testing the significant differences between the factors using Duncan's (1955) in statistical analysis of data.

Results and discussion

The results of the current study showed that the treatment T4 (64.28) was significantly superior (P≤0.05) compared to the treatments T2 (56.00) and T3 (55.68), as well as the control group T1 (50.32), whereas, did not significant differences between the two treatments T2 and T3 during the duration of cryopreservation (Table 1).The treatment T4 (57.14) outperformed all the treatments T3, T2, T1 (47.30, 47.14 and 42.14) significantly outperformed (P≤0.05), respectively, for the same characteristic after 48 hours of cryopreservation, non-significant differences between the two treatments T2 and T3, while the control treatment recorded the lowest values for individual sperm motility (Table 1).

The results of the study showed that treatment T4 (48.57) outperformed the other treatments of T2, T3 and T1 in percentage of individual sperm motility after a month of preservation by freezing, while the control treatment recorded the lowest for this characteristic, no significant differences were found for treatment T4 (42.00) after 2 months of cryopreservation compared to t T2 (40.43) and T3 (39.86 3.01), while the control treatment recorded the lowest percentage for this trait (35.12).

The reason for the superiority of treatment T4 (lyophilized vitamin E+LDL) compare the other treatments, vitamin E improves vitality and sperm motility, decrease in lipid peroxide concentrations (Bansal and Bilaspuri, 2011), also, added lyophilized LDL with vitamin E provides higher sperm capacity and protection from cold shock with the presence of non-enzymatic lipid-soluble antioxidants (Hu *et al*., 2010).

The results of the current study showed that there was a significant increase (P≤0.05) in the percentage of live sperm in the treatment T4 (72.09) compared to the treatments T2, T3 and T1 (60.93, 58.24and56.66). The treatmentss T2, T3, and T1 were not significantly different over the period of cryopreservation (Table 2).T4 (65.53) recorded the highest percentage (P≤0.05) for live sperm compared to treatments after 48 hours of cryopreservation.Whereas, there was no significant difference for T2, T3 and T1 parameters after 48 hours, with the advance of the cryopreservation, treatment T4 (61.22) outperformed all trial treatments after 1 month of cryopreservation, while the treatment T3, T1 and T2 (45.71,44.37 and 44.14), respectively, recorded the lowest percentage of live sperm.The percentage increased significantly (P≤0.05) for treatment T4 (55.70) after 2 months of cryopreservationcompare with T2, T3 and T1 (47.31and45.23).The superiority of the treatment T4 was due to the presence of the phosphate group in the lyophilized LDL, which were used in semen dilutes, the basis for protecting the plasma membrane of the sperm cell, molecules act in conjunction with the presence of vitamin E on the integrity of the plasma membrane, an increase in the percentage of live sperm from Holstein bulls (Gergatz, 2007), in addition to the

synergistic role of vitamin E with LDL in protecting fatty acids and cholesterol (Wayner*et al*., 1987).

The percentage of total sperm abnormalities decreased significantly (P≤0.05)with T4 (8.89) compared to T2, T3, and T1 (12.62,13.16 and 15.31), respectively, during the period of cryopreservation (Table 3), there were no significant differences between T2, T3 and T1 (15.46, 15.57and 20.86), respectively, in the percentage of total sperm abnormalities after 48 hours of cryopreservation, compared with treatment T4 (13.40) which had the lowest percentage of total sperm abnormalities.The percentage decreased significantly (P≤0.05)after the first month of cryopreservation with treatment T4 (18.51) compared to treatments T2, T3 and T1, non-significant differences between T2 and T3, while T4 (18.92) showed that the total sperm abnormalities were low after 2 months of cryopreservation, non-significant differences between the two treatments T2 and T3, while the control treatment was lower, reaching (26.41). During this study, it was observed that there was a significant decrease (P≤0.05) in the percentage of total distortions with treatment T4 compare the remaining treatments, due to the ability of lyophilized LDL to protect sperm, lyophilizedLDL with vitamin E in addition to the ability to combine with sperm membranes during the cooling process, which had a role in increasing her resistance to cold shock (Briand-Amirat*et al*., 2013). The superiority of treatment T4 may be due to the presence of harmful granule-free LDL, leads to stopping the breathing process of the sperm, it also works to inhibit the metabolism that is depleted by HDL (Corandin*et al*., 2013).The current study also found that cryopreservation for 48 hours, 1 month and 2 months in the presence of lyophilized LDL and vitamin E, preserved the sperm's functions with the presence of antioxidants, a reduction in the percentage of cellular abnormalities (Ana and colleagues, 2015).

Table (1) The effect of adding Lyophilized vitamin E and LDL to Tris diluent on the percentage of individual movement in Holstein bull sperm after preservation by cooling and cryopreservation for different periods.

Treatments	Cryopreservation periods			
	After cooling	After 48 hours	After 1 month	After 2 months
T1	3.51 ∓ 50.32 Ca	3.04 ∓ 42.14 Cb	3.23 ∓ 36.26 Cc	3.66 ∓ 35.12 Bc
T2	3.24 ∓ 56.00 Ba	3.72 ∓ 47.14 Bb	3.01 ∓ 42.86 Bc	3.43 ∓ 40.43 Ac
T3	2.81 ∓ 55.68 Ba	3.30 ∓47.30 Bb	3.48 ∓ 41.71 Bc	3.01 ∓ 39.86 Ac
T4	4.69 ∓ 64.28 Aa	2.53 ∓ 57.14 Ab	2.42 ∓ 48.57 Ac	2.13 ∓ 42.00 Ad

The averages bearing different capital letters within the same column and different lowercase letters within the same row are significantly different (P≤0.05), T1: Control 20% Egg Yolk T2: 5% L-LDL T3: 0.80 mmol Vitamin E T4: L-LDL + Vitamin E.L-LDL;Lyophilized-low density lipoproteins

Table (2) The effect of adding Lyophilized vitamin E and LDL to Tris diluent onthe percentage of live sperm in Holstein bull sperm after preservation by cooling and cryopreservation for different periods.

Treatments	Cryopreservation periods			
	After cooling	After 48 hours	After 1 month	After 2 months
T1	56.66∓2.81 Ba	49.57∓2.36 Bb	44.37∓2.31 Bb	43.19∓2.25 Bc
T2	60.93∓2.68 Ba	53.11∓1.71 Bb	49.14∓1.82 Bb	47.31∓1.63 Bb
T3	58.24∓1.52 Ba	51.42∓1.50 Bb	45.71∓3.29 Bc	45.23∓3.03 Bc
T4	72.09∓2.63 Aa	65.53∓1.79 Ab	61.22∓1.83 Ac	55.70∓1.76 Ac

The averages bearing different capital letters within the same column and different lowercase letters within the same row are significantly different (<0.05 P), T1: Control 20% Egg Yolk T2: 5% L-LDL T3: 0.80 mmol Vitamin E T4: L-LDL + Vitamin E.L-LDL;Lyophilized-low density lipoproteins

Table (3) The effect of adding Lyophilized vitamin E and LDL to Tris diluent onthe percentage of total abnormalities in the sperm of a Holstein bullafter preservation by cooling and cryopreservation for different periods.

Treatments	Cryopreservation periods			
	After cooling	After 48 hours	After 1 month	After 2 months
T1	15.31∓0.77 Ad	20.86∓0.39 Ac	24.51∓1.12 Ab	26.41∓0.73 Aa
T2	12.62∓0.97 Bd	15.46∓0.68 Bc	20.53∓0.81 Bb	23.16∓0.76 Ba
T3	13.16∓0.64 Bc	15.57∓0.72 Bc	20.16∓1.18 Bb	23.46∓0.81 Ba
T4	8.89∓0.83 Cc	13.40∓0.86 Bb	18.51∓0.63 Ba	18.62∓0.70 Ca

The averages bearing different capital letters within the same column and different lowercase letters within the same row are significantly different (<0.05 P), T1: Control 20% Egg Yolk T2: 5% L-LDL T3: 0.80 mmol Vitamin E T4: L-LDL + Vitamin E.L-LDL;Lyophilized-low density lipoproteins

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