Plant Archives Vol. 19 No. 2, 2019 pp. 4351-4356 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF THE ADDITION OF FREEZE-DRYING LOW-DENSITY LIPOPROTEIN TO THE TRIS DILUTION ON SOME OF THE HOLSTEIN'S SPERM TRAITS AFTER FREEZING FOR DIFFERENT DURATIONS

Ghassan S. Deharib[1] and Hazim K.J. Al-Saab[2]

[1]Animal production Department, Agriculture College, University of Al-Muthanna, Iraq.
[2]Animal production Department, Agriculture College, University of Baghdad, Iraq.

Abstract

The aim of this study was to determine the effect of replacing Freeze-drying (Lyophilization) low-density lipoprotein for different periods as an alternative to egg yolk, with different concentrations (6.4, 4.8, 3.2)g/100ml in Tris dilution, within equal periods (2h, 4h), on some Holstein bulls sperm traits. Low-density lipoproteins (LDL) were extracted from fresh egg yolk through several sequential steps, i was purified and packaged in sealed and sterile containers and kept refrigerated until by freeze-drying, low-density lipoproteins (LDLs) were filled in liquid cases in sterile cans, they were placed in a dry-freeze device. The semen was collected from 4 bulls Holstein (4-3.5 years ranging ages), using an artificial vagina with 2 ejaculation/bull/week, 1mL of semen/bull was taken to pooled semen, to remove individual differences between bulls, the liquid was divided into the eight treatments evenly (1ml/treatment) using the Tris dilator and within periods equal to (2h, 4h) the dilution ratio was 1:10, egg yolks were added by 20% to the control group for both periods, it was then added by lyophilization low-density lipoprotein with different concentrations of the six treatments (6.4) T1, (4.8) T2, (3.2) T3, within equal periods (2h, 4h), the effect of these additions was studied on some sperm traits (48 hours, 1 month, 2 months). The results of the first experiment showed that addition of (6.4) LDL was increased to a significant increase ($P \leq 0.05$) in the individual sperm movement as well as in the live sperm ratio compared with the control group, especially in the conservation period (48 hours), there was a significant superiority ($P \leq 0.05$) for the period (4h) on the period (2h) for all treatments as well as control treatment, on the other hand, there was a decrease in the percentage of sperm abnormalities for the period (4h) and for all conservation periods.

Key words: Freeze-drying, low-density lipoprotein, Tris dilution, Holstein's sperm, durations

Introduction

The technique of freezing the sperm has undergone slow development over the past decades, fertility is reduced to 50% after freezing due to decreased sperm permeability in egg membranes, as well as the low rate of movement and the high percentage of dead sperm (Nur *et al.,* 2005), the addition of dilutions to semen without knowledge of the effect of dilute type and its components leads to deterioration of the characteristics of diluted semen and low percentage of live sperm. The researchers resorted to adding glycerol or other ingredients such as egg yolks, albumin, milk and soybean extract as dilators, minimize the negative effects of freezing on sperm and may provide protection (Al-Ahmad *et al.,* 2008). The freezing process leads to a changes series in the integrity of the acrosome, the plasma membrane of the sperm and damage to the genetic material (Aitken *et al.,* 1985; Vishwanath *et al.,* 2000), the damage may occur during the freezing process to cold shock, ice crystals, oxidative stress, and change in the osmotic pressure, reorganization of lipoproteins within cell membranes (Bailey *et al.,* 2000). The continuous and free production of active oxygen species by dead, deformed and immature sperm, as well as the result of freezing and liquefaction of semen, accompanied by low concentrations of antioxidants in sperm plasma and sperm

diluents leading to an oxidative stress on the sperm cell (Sikka, 2004), the increased production of ROS also leads to lipid oxidation of the sperm cell membrane as well as damage and destruction of DNA (Sariozkan *et al.,* 2009).

The current study was conducted to determine the use of low-density lipoproteins (LDL), extracted and extracted in lyophilization of egg yolks in some of the Holstein's sperm traits.

Materials and methods

In this experiment, four bulls were trained to collect artificial semen (V) Artificial Vagina (IV) between the ages of 4-3 years and body weight between 800-600 kg/ bull, was conducted from December 2017 to January 2019, with three stages. The first phase; from December 2017 to the end of February 2018, in the laboratory of viruses Department of diagnosis, plant protection Department in the Abu Ghraib district, northwest of Baghdad, for the purpose of extracting low density lipoproteins (LDL) from fresh eggs through several sequential steps, it was purified and packaged in sealed and sterile containers and kept refrigerated until it was lyophilizated. The second phase; the experiment was in March 2018, In Al-Kendi company for vaccines and assets located in the Abu Ghraib district, where lipoproteins and LDL were filled in the case of liquid in sterile packs, it entered the dry-freeze device, It is the process of drawing moisture out of the material and turning it into dry powder, The moisture content of liquid LDL was 67%, (LDL) weight and weight after the harvest, the difference between the two weights was the loss of moisture. The third stage; was conducted at the Artificial Insemination Center in Abu Ghraib, which is affiliated to the General Company for Livestock Services (25 km west of Baghdad) from March to the end of May (2018). The semen was collected from the bulls and divided into 2 ml/ group trials and then frozen, collect semen with artificial vagina with 2 ejaculation/bull/week, the ejaculate collected number during the duration of the experiment was 28 ejaculates and the rate of 7 ejaculates/bull for the first experiment, an average of 56 ejaculates during the period of research, 2ml of semen/bull was taken to collect the spermed pool for the purpose of removing the individual differences between bulls, , After which tests were carried out to evaluate the sperm through collective movement, individual movement and the percentage of sperm live, dead and abnormalities.

Experimental data were statistically analyzed using the Completely Randomized - Design CRD, in a factorial experiment to study the effects of the coefficients for each time and the duration of conservation and overlap between them, and use of the statistical program for the ready (statistical package for the social assets) SPSS.

Results

Individual motility

The results of the present study showed a significant effect of ($P \leq 0.05$) due to reduced LDL replacements of egg yolk in semen dilution of the treatments compared to control group in the individual movement ratio of the sperm, a significant superiority of the first treatment with L-LDL (6.4%) on the rest of the treatment and control treatment after 48 hours of freezing, (42.86 ± 4.O6, 3.31 ± 52.14) within a period of (2h, 4h) (Table 1), while no significant differences were observed between the second (L-LDL 4.8) and the third (L-LDL 3.2), as well as the treatment of control within periods equal to (2h, 4h), (3.33 ± 3.23, 2.31 ± 37.14, 32.14 ± 2.83, 2.63 ± 37.14 and 32.86 ± 3.O2, 2.83 ± 37.14) respectively (Table 1), there were significant differences ($P<0.05$) for the first treatment of the individual sperm ratio of the Holstein bulls after the first month of freezing, (31.43 ± 2.76, 2.91 ± 38.57) on the second treatment within the period (2h), the third within a period of (2h, 4h) where it reached (27.86 ± 3.17 and 3.24 ± 25.71, 2.51 ± 30.00), respectively, while there were no significant differences between the first treatment and the treatment of control and within periods of (2h, 4h), (31.43 ± 2.76, 2.91 ± 38.57, 32.86 ± 3.02, 2.83 ± 37.14) respectively, as well as the second treatment within a period of (4h), reaching (3.46 ± 35.00), no significant differences in the ratio of individual movement after the second month of freezing between the first treatment and the second treatment within a period equal to (2h, 4h), (3.39 ± 29.29, 3.81 ± 26.43, 1.92 ± 30.00 ± 2.77 ± 25.00) respectively, while there were significant differences between the first treatment and the third treatment within the equivalent of (2h), (2.77 ± 25.00 and 3.31 ± 22.86) respectively, as well as with treatment of equalizer control intervals (2h, 4h), (32.86 ± 3.02, 2.83 ± 37.14), (48 hr, 1 hr and 2 hr) for the first treatment in the ratio of individual movement of the sperm within a period equal to (2h, 4h), as well as the second and third treatment and the equalization periods (2h, 4h), add the lyophilization LDL to replace the egg yolk in sperm diluents (1 month, 2 months) compared with the control treatment and within periods of 2h, 4h, 4-hour clear mental superiority of all treatments, as well as control treatment and all freezing periods.

Live sperms percentage

The results of the study showed that there were significant differences in the first treatment in live sperm ratios on the rest of the treatments, as well as control

Table 1: Effect of added LDL to TRIS dilution in percentage of individual movement of the Holstein bulls sperm after treatment and within a period of 4h, 2h and for different periods of freezing (mean ± standard error).

Treatments	Equal duration	Freezing duration		
		After 48 hours	After 1 month	After 2 month
(T1)	2h	4.06 ± 42.86 Aa	2.76 ± 31.43 Ab	2.77 ± 25.00 Bc
%6.4 Con. L-LDL	4h	3.31 ± 52.14 Aa*	2.91 ± 38.57 Ab*	1.92 ± 30.00 Bc*
(T2)	2h	3.23 ± 33.57 Ba	3.17 ± 27.86 Bb	3.81 ± 26.43 Bb
%4.8 Con. L-LDL	4h	2.31 ± 37.14 Ba*	3.46 ± 35.00 Ab*	3.39 ± 29.29 Bc*
(T3)	2h	2.83 ± 32.14 Ba	3.24 ± 25.71 Bb	3.31 ± 22.86 Cc
%3.2 Con. L-LDL	4h	2.63 ± 37.14 Ba*	2.51 ± 30.00 Bb*	2.51 ± 27.86 Bc*
(Control)	2h	3.02 ± 32.86 Ba	3.02 ± 32.86 Aa	3.02 ± 32.86 Aa
%20 Egg yolks	4h	2.83 ± 37.14 Ba*	2.83 ± 37.14 Aa*	2.83 ± 37.14 Aa*

The capital letters within one column indicate significant differences between equalization and freezing levels, small letters within one row indicate significant differences between freezing periods, * Indicates significant differences between the equal duration. L-LDL; Lyophilized Low density Lipoproteins.

treatment within the period of 2h, 4h (57.57 ± 2.58, 2.60 ± 66.61) within 48 hours of freezing (Table 2) While no significant differences were observed between the second treatment and the third treatment, as well as the treatment of control within periods equal to (2h, 4h). It was found that one month after the freezing period, the first treatment of the remaining treatments in the live sperm ratio of the Holstein bulls followed by the second and third treatments and within the period (2h, 4h) continued to reach (46.37 ± 1.81, 2.20 ± 53.84 and 43.14 ± 2.04 , 2.43 ± 52.90 and 39.79 ± 1.51, 2.63 ± 43.36), respectively. There were no significant differences between the first treatment and the control treatment (46.37 ± 1.81, 2.20 ± 53.84, 46.60 ± 2.33 ± 2.33 ± 51.50) and at intervals of 2h and 4h respectively. After 2 months, there were significant differences between the treatments and the control treatment in the live sperm ratio. The control treatment of the first, second and third treatments was higher within the period of 2h (44.90 ± 2.29 and 1.91 ± 38.91 and 39.89 ± 1.71) , 3.14 ± 34.36), respectively. While we did not notice significant differences between the first and second treatment and treatment of control within the period of (4h). There were significant differences between all treatments and treatment of control during maintenance periods (48 hours, 1 month and 2 months) and within different periods (2h, 4h). It was found that the period of storage after 48 hours of freezing was higher than the rest of the periods. A 4-hour period was found to be superior to a 2-hour period in all treatments, as well as control treatment and within different conservation periods.

Sperms head abnormalities

The results of the analysis showed significant differences in the rate of head abnormalities at freezing for 48 hours after the addition of LDL to the semen dilutions in the first, second and third treatment compared with control treatment and within the period of (2h, 4h)). The highest treatment was found in the second treatment, followed by the third treatment, followed by the first

Table 2: Effect of added LDL to TRIS dilution in percentage of Live sperms percentage of the Holstein bulls sperm after treatment and within a period of 4h, 2h and for different periods of freezing (mean±standard error).

Treatments	Equal duration	Freezing duration		
		After 48 hours	After 1 month	After 2 month
(T1)	2h	2.58 ± 57.57 Aa	1.81 ± 46.37 Ab	1.91 ± 38.91 Bc
%6.4 Con. L-LDL	4h	2.60 ± 66.61 Aa*	2.20 ± 53.84 Ab*	1.70 ± 43.31 Ac*
(T2)	2h	2.49 ± 48.11 Ba	2.04 ± 43.14 ABb	1.71 ± 39.89 Bc
%4.8 Con. L-LDL	4h	2.71 ± 58.42 Ba*	2.43 ± 52.90 Ab*	1.81 ± 49.31 Ac*
(T3)	2h	1.63 ± 45.14 Ba	1.51 ± 39.79 Bb	3.14 ± 34.36 Bc
%3.2 Con. L-LDL	4h	2.08 ± 52.04 Ca*	2.63 ± 43.36 Bb*	2.81 ± 39.22 Bc*
(Control)	2h	2.08 ± 49.53 Ba	2.33 ± 46.60 Ab	2.29 ± 44.90 Ab
%20 Egg yolks	4h	1.96 ± 56.41 BCa*	2.33 ± 51.50 Ab*	2.13 ± 48.70 Ab*

The capital letters within one column indicate significant differences between equalization and freezing levels, small letters within one row indicate significant differences between freezing periods, *Indicates significant differences between the equal duration. L-LDL; Lyophilized Low density Lipoproteins.

treatment within (2h), where it reached (8.45 ± 0.58, 7.80 ± 0.47 and 7.20 ± 0.35), respectively (Table 3). There were significant differences (Pd"0.05) in the rate of head deformity of the sperm of the Holstein bulls after the first month of freezing. The second treatment and the third treatment were superior in the rate of head deformities on the first treatment within a period of (2h, 4h) 0.52, 7.78 ± 0.53, 9.67 ± 0.60, 7.46 ± 0.51 and 8.91 ± 0.64, ± 7.00 ± 0.36), respectively (Table 11). While the highest percentage of deformities in the treatment of control and within the period of (2h), which amounted to (10.85 ± 0.51).

There were significant differences (R05.05) in the rate of head deformities after the second month of freezing. The control treatment in the rate of head deformities exceeded all treatments within a period of 2h (11.27 ± 0.52). The lowest treatment was the first treatment followed by the third treatment within the period (4h) at (7.31 ± 0.33 and 8.47 ± 0.49) respectively (Table 11). There were significant differences between freezing periods within the single treatment as well as control treatment. It was also observed that a period of (4h) was superior to obtain the lowest rate of head-to-head deformities in all treatments as well as in control treatment.

Total sperm abnormalities

A significantly affect (P<0.05) in total malformation as a result of the addition of LDL to the diluted (Tris) when frozen and for different periods. The results showed that after 48 hours of freezing, the ratio of total deformities in the first treatment was reduced within 2h and 4h (22.66 ± 0.78, 17.44 ± 0.70). (26.47 ± 0.97, 20.43 ± 0.74 and 26.13 ± 0.95, 19.59 ± 0.73), respectively, while the control treatment was superior in the highest percentage of sperm defects on all experimental factors (2h, 4h) at 30.53 ± 0.70, 23.86 ± 0.81 (Table 14). On the other hand, the results showed that after the first month of freezing, there were significant differences between the treatments and the control treatment. The lowest treatment was found in the first treatment within the period (2h, 4h) with 27.47

Table 3: Effect of added LDL to TRIS dilution in Sperms head abnormalities percentage of the Holstein bulls sperm after treatment and within a period of 4h, 2h and for different periods of freezing (mean ± standard error).

Treatments	Equal duration	Freezing duration		
		After 48 hours	After 1 month	After 2 month
(T1)	2h	0.35 ± 7.20 Cb*	0.64 ± 8.91 Ba*	0.5 ± 9.50 Ca*
%6.4 Con. L-LDL	4h	0.38 ± 5.37 Bb	0.36 ± 7.00 Ca	0.33 ± 7.31 Ca
(T2)	2h	0.58 ± 8.45 ABb*	0.52 ± 9.67 ABa*	0.55 ± 10.47 Ba*
%4.8 Con. L-LDL	4h	0.45 ± 6.55 Cb	0.53 ± 7.78 ABa	0.44 ± 8.86 ABa
(T3)	2h	0.47 ± 7.80 BCb*	0.60 ± 9.67 ABb*	0.57 ± 10.55 Ba*
%3.2 Con. L-LDL	4h	0.35 ± 5.83 Bc	0.51 ± 7.46 BCb	0.49 ± 8.47 BCa
(Control)	2h	0.46 ± 9.43 Ab*	0.51 ± 10.85 Aa*	0.52 ± 11.27 Aa*
%20 Egg yolks	4h	0.40 ± 7.33 Ab	0.56 ± 8.84 Aab	0.48 ± 9.53 Aa

The capital letters within one column indicate significant differences between equalization and freezing levels, small letters within one row indicate significant differences between freezing periods, *Indicates significant differences between the equal duration. L-LDL; Lyophilized Low density Lipoproteins.

Table 4: Effect of added LDL to TRIS dilution in Total sperm abnormalities percentage of the Holstein bulls sperm after treatment and within a period of 4h, 2h and for different periods of freezing (mean ± standard error).

Treatments	Equal duration	Freezing duration		
		After 48 hours	After 1 month	After 2 month
(T1)	2h	0.78 ± 22.66 Cb*	1.02 ± 27.47 Ca*	0.76 ± 28.71 Ca*
%6.4 Con. L-LDL	4h	0.70 ± 17.44 Cb	0.87 ± 21.41 Ca	0.74 ± 22.57 Ca
(T2)	2h	0.97 ± 26.47 Bb*	0.78 ± 31.39 Ba*	0.83 ± 32.77 Ba*
%4.8 Con. L-LDL	4h	0.74 ± 20.43 Bc	0.84 ± 24.56 Bb	0.74 ± 27.10 Ba
(T3)	2h	0.95 ± 26.13 Bb*	1.13 ± 30.36 Ba*	0.95 ± 32.87 Ba*
%3.2 Con. L-LDL	4h	0.73 ± 19.59 Bb	1.17 ± 24.10 Ba	0.82 ± 26.43 Ba
(Control)	2h	0.70 ± 30.53 Ab*	0.81 ± 34.87 Aa*	0.75 ± 36.53 Aa*
%20 Egg yolks	4h	0.81 ± 23.86 Ab	0.88 ± 28.51 Aa	0.70 ± 30.46 Aa

The capital letters within one column indicate significant differences between equalization and freezing levels, small letters within one row indicate significant differences between freezing periods, * Indicates significant differences between the equal duration. L-LDL; Lyophilized Low density Lipoproteins.

± 1.02, 21.41 ± 0.87 (Table 4). After the second month of freezing, significant differences were found between the treatments and the treatment of control, the highest percentage of total deformities in the treatment of control and the lowest proportion of the first treatment within periods of 2h, 4h. As for the different periods of freezing, there was a significant effect between the coefficients as well as the treatment of control. There was also a significant difference between the equalization periods, with a period of 2 hours exceeded the total deformity rate over a period of 4 hours and all transactions.

Discussion

The benefit of using Lyophilized LDL derived from egg yolks is that this material has the ability to protect against cold shock damage and has improved fertility capacity (Anton, 2007), isolate and purify the LDL from the egg yolk, and then use it as a base material (Moussa *et al.,* 2002), in this study, Lyophilized LDL was added with different concentrations (6.4, 4.8, 3.2)g/100 ml in the dilution solution and the same sperms, (48 hours, 1 month, 2 months) as an alternative to a full egg yolk, Showing his active role in activating the sperms. In this study, (6.4%) Lyophilized LDL was shown to have improved the cell characteristics of these frozen sperm, led to the superiority of most qualities in the percentage of individual movement, another advantage of using Lyophilized LDL is the low percentage of mutated sperm in the Holstein rats compared to the control treatment, this is due to the importance of separation of the particles in the egg yolk responsible for protection of the sperm from the impact of cold shock, for use in solutions dilute the semen of many animals, such as bulls (Moussa *et al.,* 2002), dogs (Bencharif *et al.,* 2008), pigs (Jiang *et al.,* 2007), goats (Al-Ahmad *et al.,* 2008) and horses (Pillet *et al.,* 2011). The reasons for the increase in the sperm percentage of the safe because the molecules of this material protective role to protect sperm cells from the potential harmful effects during the methods of work and preparation, as these molecules follow mechanisms to achieve stability in the proportion of safety membrane plasma (Gergatz, 2007), these were all of the following: The first; Isolation and stopping the effectiveness of proteins in the sperm plasma Binder of sperm protein (BSP), LDL molecules interact with these proteins by interacting with a complex component (LDL-) (BSP) proteins, which in turn prevents BSP from merging with the cell's plasma membrane and causing these proteins to damage and damage to the surface Cell by pulling and adsorption of cholesterol and phospholipids, this fast interactive mechanical remains constant and is not affected by temperature change during conservation because it has the ability to achieve stability in the structure of the plasma membrane (Manjunath *et al.,* 2002). Second; it shows that the return phospholipids of the LDL molecules compensate for the loss of components. This reduces the sensitivity of the sperm cells to the negative effect during the exposure of the cryinjery caused by the low temperature during changes in the cell membrane during the freezing phase. The change in cycle led to the transformation of the nature of the clay into the crystalline state (Graham and Foot, 1987). Third; The partial LDL is damaged and destroyed during the process of freezing, resulting in the release of phospholipids and cholesterol to be a film thin layer on the outer surface of the plasma membrane of the sperm (Quinn and Chapman, 1980).

References

Aitken, R.J., M. Sutton, P. Warner and D.W. Richardson (1985). Relationship between the movement characteristics of human spermatozoa and their ability to penetrate cervical mucus and zona free hamster oocytes. *J. Reprod. Fertil.,* **73:** 441–449.

Al-Ahmad, A.M.Z., G. Chatagnon, L. Amirat-Briand, M. Moussa, D. Tainturier and M. Anton (2008). Use of glutamine and low density lipoproteins isolated from egg yolk to improve buck semen freezing. *Reprod. Domest. Anim*, **43:** 429-36.

Anton, M. (2007). Low-density Lipoproteins (LDL) or Lipovitellenin Fraction. In: Bioactive Egg Compounds. Huopalahti, R., R. Lopez–Fandino, M. Anton and R. Schade (Ed.). Springer-Verlag, Berlin Heidelberg, Germany. p.7.

Bailey, J.L., J.F. Bilodeau and N. Cormier (2000). Semen cryopreservation in domestic animals : A damaging and capacitating phenomenon. *J. Androl*, **21:** 1-7.

Bencharif, D., L. Amirat, M. Anton, E. Schmitt, S. Desherces, G. Delhomme, M.L. Langlois, P. Barriere, M. Larrat and D. Tainturier (2008). The advantages of LDL (Low Density Lipoproteins) in the Cryopreservation of canine semen. *Theriogenology*, **70:** 1478–1488.

Gergatz, E. (2007). The artificial insemination in sheep; in Artificial Insemination in domestic mammals; Szerk. (Edited) Tamas Pecsi; Pecsi Tamas, Mezogazda Kiado, Bp. ISBN 978-963-286-237-8.

Graham, J.K. and R.H. Foote (1987). Effect of several lipids, fatty acyl chain Length, and degree of unsaturation on the motility of bull Spermatozoa after cold shock and freezing. *Cryobiology*, **24:** 42-52.

Jiang, Z.L., Q.W. Li, W.Y. Li, J.H. Hu, H.W. Zhao and S.S. Zhang (2007). Effect of low density lipoprotein on DNA integrity of freezing–thawing boar sperm by neutral comet assay.

Anim. *Reprod. Sci.*, **99:** 401–407.

Manjunath, P., V. Nauc, A. Bergeron and M. Menard (2002). Major Proteins of bovine seminal plasma bind to the low-density lipoprotein fraction of hen's egg yolk. *Biol. Reprod.*, **67:** 1250-1258.

Moussa, M., V. Martinet, A. Trimeche, D. Tainturier and M. Anton (2002). Low density lipoproteins extracted from hen egg yolk by an easy method : cryoprotective effect on frozen - thawed bull semen. *Theriogenology*, **57:** 1695-1706.

Nur, Z., I. Dogan, U. Gunay and M.K. Soylu (2005). Relationships Between sperm membrane integrity and other semen quality characteristics of the semen of Saanen goat bucks. *Bull Vet Inst Pulawy.*, **49:** 183-187.

Pillet, E., G. Duchamp, F. Batellier, V. Beaumal, M. Anton, S. Desherces, E. Schmitt and M. Magistrini (2011). Egg yolk plasma can replace egg yolk in stallion freezing extenders. *Theriogenol.*, **75:** 105-114.

Quinn, P.J. and D. Chapman (1980). The dynamics of membrane structure. CRC. *Crit Rev Biochem.*, **8:** 1-117.

Sariozkan, S., M.N. Bucak, P.B. Tuncer, P.A. Uiutas and A. Bilgen (2009). The influence of cysteine and taurine on microscopic oxidative strees parameters and fertilizing ability of bull semen following cryopreservation. *Cryobiology*, **58:** 134- 138.

Sikka, S.C. (2004). Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. *J. Androl.,* **25:** 5-18.

Vishwanath, R. and P. Shannon (2000). Storage of bovine semen in liquid and frozen state. *Anim Reprod Sci.*, **62:** 23–53.