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The Effect of Thawing Duration and Different Temperatures in the Vitality of Semen Preserved by Freezing for Five Years in Holstein Bulls

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Abstract
This study was carried out in the artificial insemination center of the Department of Livestock in the Abu Ghraib region/Ministry of Agriculture (25 km west of Baghdad) and the Animal Physiology Laboratory at the College of Agriculture, University of Diyala in February 2024, using sperm preserved by freezing for five years from five Holstein bulls, ages ranging from 2.5-3 years old and with a body weight ranging between 500-750 kg/bull. The experiment was divided into three treatments: T1, thawing straws of frozen semen at a temperature of 37°C and a thawing duration of 30 seconds. T2, thawing straws of frozen semen at a temperature of 50°C and a thawing duration of 12 seconds. T3, thawing straws of frozen semen at a temperature of 60°C and a thawing duration of 6 seconds. The characteristics were studied after preservation by freezing for five years (percentage of individual motility, live and dead sperm,%, integrity of the plasma membrane and acrosome,%, percentage of deformities in the head, midsection, and tail, total deformities, and percentage of normal sperm), where the results showed in all the characteristics studied the moral and mathematical superiority of the first treatment, T1, compared to With the second transaction T2 and the third transaction T3. On the other hand, the second treatment, T2, excelled morally and mathematically compared to the third treatment, T3.

Keywords: Holstein, acrosome, dead sperm

Introduction

Semen freezing is the most widely used technology in the world to spread genetic traits in farm animals on a large scale (Oliveira *et al*., 2013). Semen has been preserved by freezing (semen cryopreservation) for more than half a century for the purposes of artificial insemination (Calisici, 2010). The process of preserving semen by freezing is considered one of the most efficient methods at the present time because it maintains the integrity of the sperm and prolongs its storage period, thus increasing the rate of fertilization and pregnancy when used for artificial insemination purposes (Lemma, 2011; Crespilho *et al*., 2014). The oxidative stress resulting from the freezing and liquefaction processes of semen is often accompanied by a decrease in the concentration of antioxidants in the seminal plasma, and thus causes a decrease in the levels of enzymatic and non-enzymatic defense molecules and enzymatic defenses such as catalase and Super. Superoxide dismutase (SOD; superoxide dismutase) and glutathione peroxidase (GPx; glutathione peroxidase) and non-enzymatic enzymes such as vitamin C and E in seminal plasma (Aitken and Baker, 2004; Sikka, 2004; Taşdemir *et al*., 2013). Which leads to changes in the plasma membrane and a change in the activity of proteins, and then a change in the permeability of water and solutes and a loss of sperm viability (Purdy *et al*., 2010). The process of liquefaction of semen tubes is of similar importance to the process of cryopreservation in terms of its effect on preserving the functional activity of the sperm, because the process of liquefaction restores the life of the sperm as well as its physiological activity and ability to fertilize. Therefore, the process of liquefaction must be carried out carefully to preserve the quality of the semen. Chatterjee *et al*., (2017).

It is believed that the quality of semen after liquefaction is affected by many factors during the cryopreservation process, such as the type of diluent, glycerol concentration, packaging method, and the rate of freezing and liquefaction (Robbins *et*

al., 1976; Rastegarnia *et al*., 2013). In a study conducted by Al-Badry (2012) to demonstrate the effect of liquefaction time on some characteristics of semen, with different liquefaction durations (30 seconds, 2, 4 hours), it showed a significant increase in the percentage of individual motility, the integrity of the plasma membrane, the percentage of acrosome integrity, and live sperm, and a significant decrease in the percentage Percentage of total deformations for the duration of liquefaction (30 seconds) compared to 2 and 4 hours at 30°C. Rastegarnia *et al*. (2013) also showed that the percentage of individual motility of buffalo sperm decreased significantly (P≤0.05) when the duration of asking was increased (50 and 70 seconds) compared to the asking duration of 30 seconds/37°C. While they did not find any significant difference in the percentage of live sperm or the integrity of the membrane. Plasma and acrosome integrity when increasing the liquefaction duration (30, 50 and 70 seconds/37°C). This was confirmed by the results of Abdulkareem (2014), who used different liquefaction durations (15, 30, 60 and 120 minutes) after cryopreservation for a full year of semen, as he found the highest percentage of motility. Individual semen and live sperm for 15 and 30 minutes of estrangement at a temperature of 30°C compared to the rest of the estrangement periods for sperm from Holstein bulls. The aim of this study is to determine the effect of temperature and different estrangement durations on the viability of frozen fluid for a period of five years in Holstein bulls.

Materials and Methods

Semen stems from Holstein bulls preserved in liquid nitrogen at a temperature of 196°C for five years were selected and were subjected to three treatments with different temperatures and liquefaction times according to the following treatments:

1. The first treatment (T1) used a temperature of 37°C and a liquefaction period of 30 seconds
2. The second treatment (T2) used a temperature of 50°C and a liquefaction period of 12 seconds
3. The third treatment (T3) used a temperature of 60°C and a liquefaction period of 6 seconds

Individual motility was estimated according to what was stated by Walton (1933), whereby a drop of diluted semen was placed on a glass slide at a temperature of 37°C with the addition of three drops of sodium citrate at a concentration of 2.9%, for fresh semen, while for semen The stalk is stored in tubes, so the stalk is cut at both ends, neglecting the first drop and then relying on the second drop. Then the cover of the slide is placed on the sample and it is examined under a microscope with an objective lens with a magnification of the movement was converted to percentages to facilitate statistical analyses.

The percentage of live sperm was calculated according to what was stated by Swanson and Beardon (1951), whereby a drop of frozen semen was taken and placed on a clean glass slide at a temperature of 37 °C, then one drop of a mixture of (5% eosin dye and 10% necrocin) was added to it. Then I made a smear on another glass slide at an angle of 45 degrees, and examined it under a microscope with a magnification of 200 sperm were counted in different fields of the slide, after which the percentage of live sperm was estimated according to the following equation:

Percentage of live sperm% = (number of live sperm)/200x100

The percentage of deformed sperm was calculated according to the method of Hancock (1951) on the same slide for estimating the percentage of live sperm. It was examined with a microscope under 40x magnification and the deformed sperm were identified by counting 200 sperm in different fields of the slide. The studied deformities were classified into deformities specific to the head. The sperm is the giant head, the dwarf, the tapering, the narrow (narrow), the detached head, the swollen midpiece, the twin midpiece, and the upper protoplasmic droplet, which is related to the tail. Twin tail and coiled tail. After that, the percentage of deformed sperm was estimated for the head, the middle part, the main part, and the final part for each deformity, and the total deformities according to the following equation:

Percentage of total sperm deformities% = (number of deformed sperm)/200x100

Freezing ability was estimated according to the method of Patt and Nath (1969) to determine one of the characteristics of semen after cryopreservation, which is its ability to maintain its physiological properties, and after freezing it was calculated as in the following equation:

$$\text{Freezability\%} = \frac{\text{The rate of individual movement of the frozen liquid}}{\text{Percentage of individual movement of fresh liquid}} \text{ x } 100$$

According to the method of Jeyendran (1984), the percentage of sperm with an intact plasma membrane was estimated by placing 10 micromol of semen in a test tube and then adding a hypo-osmotic solution (fructose 8.72 g/L and sodium citrate 4.74 g/L), which reaches the osmotic pressure. (100 mOsm/L), pH 8.00, and placed in a water bath for 60 minutes at a temperature of 37°C. The sample was then examined under a microscope at a magnification of slide, and then the percentage of sperm with an intact plasma membrane was calculated, and the percentage of sperm with an intact plasma membrane was calculated according to the following equation:

Healthy sperm, plasma membrane% = number of healthy sperm/number of total sperm x 100

Acrosome integrity = number of sperm Acrosome intact/200x100

The data were analyzed statistically using the statistical program SAS (Statistical Analysis System) (2010) using the General Linear Model (GLM) method. Testing for significant differences between means was conducted using Duncan's multinomial test (Duncan, 1955).

$Yij = \mu + Ti + eijk$

Yij = the value of the ith observation due to the jth focus

m = the general average of the studied trait.

Ti = effect of concentration and duration, as the value of i = 3 (dissolution temperature is 30 and a dissolution period is 30 seconds, dissolution temperature is 50 and a dissolution period is 12 seconds, dissolution temperature is 60 and a dissolution period is 6 seconds)

eijk = experimental error that is normally and independently distributed with a mean of zero and an equal variance of $\epsilon 2\ \sigma$

Results and Discussion

Figure No. (1) shows that the collective and individual movement of fresh semen in bulls No. 3 and No. 5 is a mathematical difference compared to bull Nos. 1, 2 and 4, and

the higher the percentage of collective and individual movement, this indicates the vitality of the semen and also reflects positively in Fertilization rate.

Figure No. (2) Shows the individual movement after cooling at a temperature of 5°C, and the individual movement after freezing. There is a mathematical difference for bull no. (3) and No. (5) compared to other bulls, and this is reflected positively when cows are artificially inseminated, and on the other hand, it indicates natural antioxidants, which It has an important role in interacting directly with the free radicals produced during freezing.

The results of the analysis of variance showed that there is a clear mathematical and moral difference for bull No. 3 and 5 in Figure No. (3) Compared to the rest of the bulls after preservation by freezing for five years. The reason may be due to the role of natural antioxidants, which have an important role in interacting directly with free radicals. Thus, sperm vitality is maintained through cryopreservation, and this mathematical difference coincides with the collective and individual movement of fresh semen.

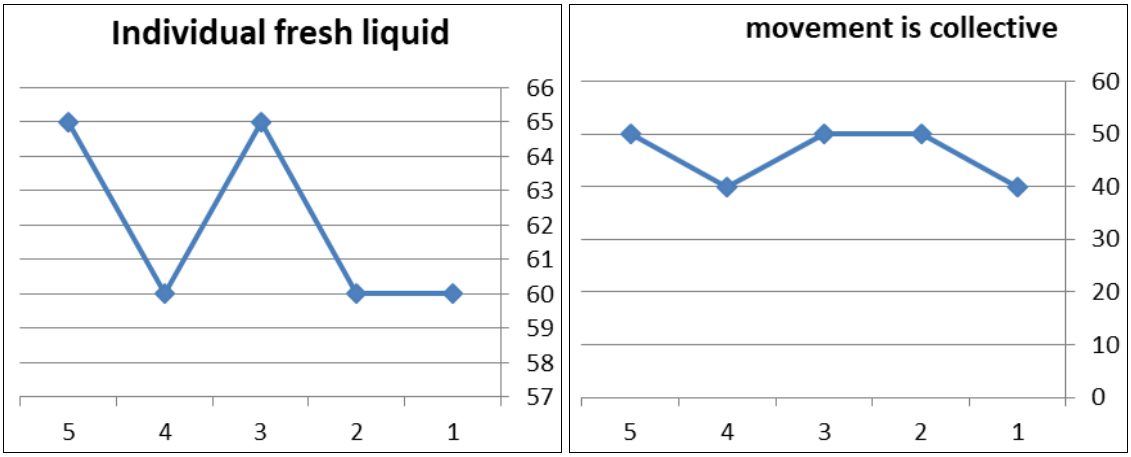

Fig 1: Shows the collective and individual movement of fresh semen (where 1,2,3,4,5 each number represents a bull)

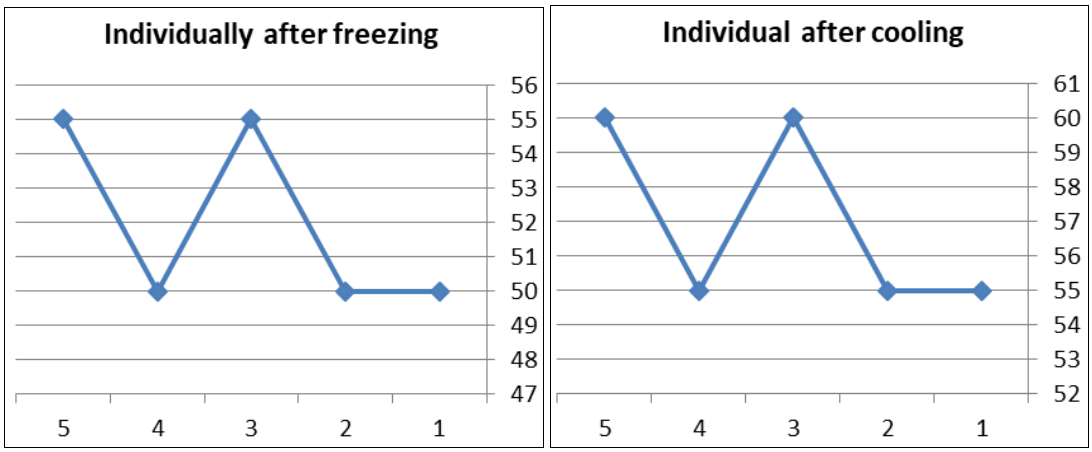

Fig 2: Shows the individual movement with a cooling kit at 5°C and the individual movement after freezing

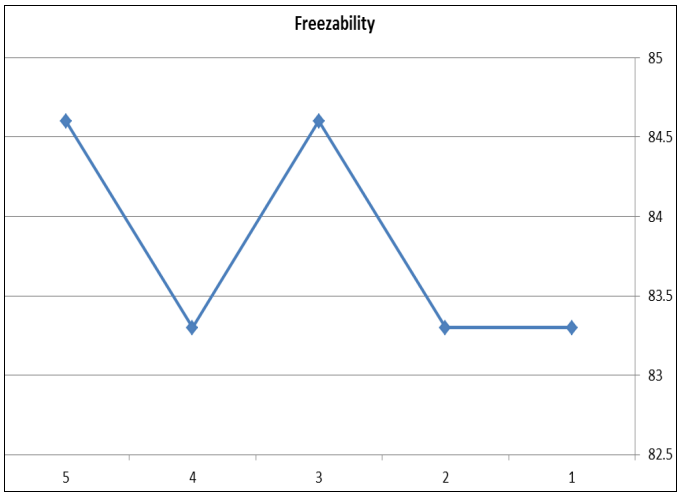

Fig 3: Shows the relationship between the five bulls' susceptibility to freezing and the percentages of individual sperm motility

The results of Table (1) show that the effect of the duration of liquefaction and the different temperatures on the percentage of individual motility, the percentage of live sperm, the percentage of dead sperm, the percentage of plasma membrane integrity, and the percentage of acrosome integrity in the tracheas of Holstein bulls preserved by freezing for five years was superior to the first treatment, T1. Morally and mathematically compared to the second treatment, T2, and the third treatment, T3. On the other hand, the second treatment, T2, excelled morally and mathematically compared to the third treatment, T3. This may be due to the fact that the best temperature is 37°C, with a thawing period of 30 seconds, to obtain the highest vital percentage of semen, and this reflects positively. When cows are artificially inseminated in order to obtain the highest fertilization rate, the more the frozen tracheas are exposed to a higher temperature, it negatively affects the vitality of the semen and thus affects the fertilization rate. The results agreed with Abdulkareem (2014). On the one hand, the reason may be that it is believed that the quality of semen after liquefaction is affected by many factors during the cryopreservation process, such as the type of diluent, glycerol concentration, method of filling, rate of freezing and liquefaction (Robbins *et al*., 1976; Rastegarnia *et al*., 2013). This study has shown that different temperatures and durations of liquefaction lead to damage to the integrity of the plasma membrane and the integrity of the acrosome, and this reflects negatively on the vitality of semen in Holstein bulls.

Table 1: Shows the effect of the duration of thawing and different temperatures on individual motility, live and dead sperm, and the integrity of the plasma membrane and semen acrosome preserved by freezing for five years in Holstein bulls

Treatment	Individual Movement%	Live Sperm%	Dead Sperm%	Plasma Membrane Integrity%	Acrosome Safety%
T1	52.00±1.22 a	75.00±2.23 a	2.00±25.00 a	1.24±76.20 a	1.87±81.00 a
T2	47.00±1.22 b	68.60±0.97 b	0.75±31.4 a	1.87±71.00 a	1.87±76.00 a
T3	39.00±1.87 c	61.00±1.00 c	0.90±39.00a	1.22±57.00 a	1.58±65.00 b

T1 Thaw frozen semen tubes at a temperature of 37 and a thawing time of 30 seconds.
T2 Thawing frozen semen tubes at a temperature of 50°C and a thawing time of 12 seconds.
T3 Thawing frozen semen tubes at a temperature of 60°C and a thawing time of 6 seconds.
Different letters within one column indicate a significant difference p≤0.05

The results of Table (2) showed that the percentage of deformities of the head, midsection, tail, total deformities, and percentage of normal sperm were significantly and mathematically superior to the first treatment, T1, compared to the second treatment, T2, and the third treatment, T3. On the other hand, the second treatment, T2, was superior compared to the third treatment, T3, and this may be attributed to The reason is that when the melting temperature rises, the rate of deformations increases, and this is what happened in the second and third treatments. The more deformities increased, it led to a decrease in fertilization in artificially inseminated cows. On the other hand, the reason for high temperatures is that they cause denaturation of proteins, which affects the properties of living cells. On the other hand, it came These results are in conjunction with individual motility, the percentage of live and dead sperm, the integrity of the plasma membrane, and the integrity of the acrosome. On the other hand, the process of liquefaction of semen tubes is of similar importance to the process of cryopreservation in terms of its effect in preserving the functional activity of the sperm, because the process of liquefaction restores the life of the sperm as well as its physiological activity and ability to fertilize, so the process of liquefaction must be carried out carefully to preserve the quality of the semen. Chatterjee *et al*., (2017).

Table 2: Shows the effect of the duration of thawing and different temperatures on deformities of the head, segment, midsection, and tail, total deformities, and the percentage of normal sperm in Holstein bulls preserved by freeze for five years

Treatment	Head Deformities%	Midsegment Deformities%	Tail Deformities%	Total Deformities%	Normal Sperm%
T1	6.60±0.24 a	0.60±0.24 a	7.80±0.37 a	15.20±0.58 a	0.50±84.8 a
T2	6.40±0.24 b	1.60±0.24 a	7.00±0.31 b	16.80±0.37 a	0.30±83.2 a
T3	8.60±0.24 b	1.40±0.24 b	8.40±0.24 b	17.60±0.24 b	0.20±82.4 b

T1 Thaw frozen semen tubes at a temperature of 37 and a thawing time of 30 seconds.
T2 Thawing frozen semen tubes at a temperature of 50°C and a thawing time of 12 seconds.
T3 Thawing frozen semen tubes at a temperature of 60°C and a thawing time of 6 seconds.
Different letters within one column indicate a significant difference p≤0.05

Reference

1. AbdulKareem TA. Influence of thawing period on some post-thaw semen characteristics of Holstein bulls following catalase addition to Tris extender. Al-Anbar J. Vet. Sci. 2014; 7(1):22-28.
2. Aitken R, Jand MA Baker. Oxygene stress and male reprod-uctive biology. Reprod. Fertil. Dev. 2004; 16:581-588.
3. Al-Badry KI. Effect of various thawing times and temperatures on frozen semen quality of Friesian bulls in Iraq. Inter. J. Anim. Vet. Adv. 2012; 4(6):384-388.
4. Calisici O. Investigation of antioxidative capacity in bovine seminal-plasma: Effects of omega 3 fattyacids. PhD. Thesis, College of Samaun/Türkei, 2010.
5. Chatterjee T, Sontakke SH, Khadse J. Efficiency of thawing cum controller unit against normal water bath for determining the cattle sperm quality parameter test.International Journal of Veterinary Sciences and Animal Husbandry. 2017; 2(2):20-22.
6. Crespilho AM, Nichi M, Guasti PIV, Freitas-Dell CP, Safilho MF, Mazlero RR, *et al*. Sperm fertility and viability following 48h of refrigeration: Evaluation of different extenders for the preservation of bull semen in liquid state. Anim. Reprod. Sci. 2014; 146:126-133.
7. Duncan DB. Multiple Range and Multiple F tests. Biometrics. 1955; 11:1-42.

8. Hancock JL. Astaining technique for the study of temperature shock in seme Nature Land. 1951; 167:323-32
9. Jeyendran RS. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to the other sperm characteristics, J. Reprod. Fert. 1984; 70:219-28.
10. Lemma A. Effect of cryopreservation on sperm quality and fertility. In: Artificial Insemination in Farm Animals. M. Manafi (ed.). Chapter 12. InTech Open Access Publisher, 2011, 307-312.
11. Oliveira LZ, Monteiro FM, RP.de Arrudaand ECC. Celeghini. The importance of semen quality in AI programs and advances in laboratory analyses for semen characteristics assessment. In: Success in Artificial Insemination- Quality of Semen and Diagnostics Employed. A. Lemma (ed.), InTech Open Access publisher, 2013, 1-16.
12. Patt JA, Nath J. Effects of diluents, equilibration time, and freezing rates on the storage of ram semen. Cryobiology. 1969; 5:385-392.
13. Purdy PH, Mocé E, Stobart R, Murdoch WJ, Moss GE, Larson B. *et al*. The fertility of ram sperm held for 24h at 5 degrees C prior to cryopreservation. Anim. Reprod. Sci. 2010; 118:231-235.
14. Rastegarnia A, Shahverdi A, Topraggaleh TR, Ebrahimi B, Shafipour V. Effect of different thawing rates on post-thaw viability, kinematic parameters and chromatin structure of buffalo (Bubalus bubalis) spermatozoa. Cell J. 2013; 14:306-313.
15. Sikka SC. Andrology lab corner: Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. Journal of Andrology. 2004; 25:5-18.
16. Swanson EW, Beardon HJ. An eosin nigrosin stain differentiating live and dead bovine spermatozoa. J. Anim. Sci. 1951; 10:981-987.
17. Taşdemir U, Buyukleblebici S, Tuncer APB, Coskun E, Ozgurtas T, Aydın FN. *et al*. Effects of various cryoprotectants on bull sperm quality, DNA integrity and oxidative stress parameters. Cryobiology. 2013; 66:38-42.
18. Walton A. Technique of artificial insemination. Mp. Bur. Anim. Genet. 56. Iiius- Edinburgh, 1933.