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# EFFECT OF ADDITION L-ARGININE TO EXTENDER OF POOR MOTILE HOLSTEIN BULLS SPERMATOZOA.

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## ABSTRACT

*This study was carried out to investigate the effect of addition of different levels of L-arginine (0, 3, 5, 7 and 10 mM) to improve the poor motile of Holstein bulls spermatozoa. Semen collected from five Holstein bulls once weekly and ejaculates with 45-50% mass motility was pooled. Semen was diluted with Tris- egg yolk extender at 37C° and divided into five parts,1st part without supplement (control), other four parts 2nd , 3rd , 4th and 5th were supplemented with 3, 5, 7 and 10 mM L-arginine respectively. Diluted semen was equilibrated at 4 C° for 4 h, then, filled in 0.25 ml French straws. Straws were kept on liquid nitrogen vapors for 10 min and then plunged and stored in liquid nitrogen at -196C°. Progressive sperm motility, live sperm, abnormal sperm and sperm acrosome integrity were determined post-dilution, equilibration and post-thawing processing.*

*The results were referred that extenders with concentrations of 5 and 7mM L-Arginine were improved all parameters studied than 3; 10 mM L-arginine extenders and control extender at different cryopreservation processes. The percentage of DNA damage were 20.0, 10.8, 4.40, 3.20 and 9.40 for control and 3, 5, 7 and 10 mM L-arginine extenders respectively. Significant differences in chromatin integrity were observed among control and treatments (P<0. 05). Also, fertility rate was higher in Friesian cows artificially inseminated with 5 and 7 mM L-arginine (65 and 66%) compared with control (45%).*

***Conclusively,*** *it could be concluded that adding L-arginine with concentrations of 5 or 7mM to Tris-extender improved the freezability and fertility of poor quality of Holstein bull spermatozoa.*

**Key words:** L- Arginine, Semen extender, Poor quality Semen, DNA damage, Holstein bull

## INTRODUCTION

Sperm production is an important factor influencing the reproductive capacity of males of economically important species (Berndtson, 2008). The application of artificial insemination (AI) has been shown to have the ability to disseminate genes from super genetic males for improving productive performance (El-Sheshtawy *et al.*, 2015).

Successful AI relies on successful extender that preserve the functional activity of spermatozoa (viability and fertilizing ability) during storage at different temperatures, because during preservation several factors may be responsible for the possible decrease of fertilizing ability of spermatozoa (Anand, 1979), The composition of the extender in which semen is diluted before freezing is one of the most important factors affecting cryopreservation (El-Sheshtawy *et al.*, 2015). Therefore, various additives have been incorporated into semen extenders to improve sperm motility and fertility (Entesar, 2015). Cryopreservation induces damage to spermatozoa that may result in loss of motility, plasma membrane integrity, and fertilizing capacity (Aitken *et al.*, 1998). Freezing and thawing of bull semen leads to a decrease in percentage of intact sperm, reducing the percentage of viable sperm cells (Woeleder *et al.*, 1997). It has yet some adverse effect on the spermatozoa manifested as a depression in viability rate, structural integrity, depressed motility and conception rates (Watson, 2000; Batellier *et. al.*, 2001; Medeiros *et al.*, 2002).

Mammalian spermatozoa have very specific lipid composition and a high ration of polyunsaturated fatty acids. This unusual composition of the sperm membrane is responsible for its flexibility and the functional job of sperm cells. However, the lipids of spermatozoa are the main cause of peroxidation, which may cause severe functional disorders of sperm. Oxidative stress can cause pathological lipid peroxidation of the sperm membrane (Sanocka and Kurpisz, 2004).

L-arginine is amino acid plays an important role in stimulating sperm motility in rabbits *(*Radany *et al.*, 1981), humans *(*Aydin *et al.*, 1995*)* and goats *(*Patel *et al.*, 1998*)* under in vitro conditions. L-arginine prevents membrane lipid peroxidation in spermatozoa under different peroxidation conditions (Srivastava *et al*., 2006, Evan *et. al.*, 1990). It has been proposed that the beneficial effects of L-arginine are linked to nitric oxide (NO) (Chemineau, *et al.*, 1991). It has shown that L-arginine improves the rate of glycolysis, resulting in higher rates of ATP and lactate generation in spermatozoa (Aydin *et al*., 1995).

Therefore, this study was conducted as attempt to know the freezability and conception rate of poor Holstein spermatozoa after addition of L-Arginine to semen extenders.

## MATERIALS AND METHODS

This study carried out at the International Livestock Management Training Center (ILMTC), Sakha Station, Animal Production Research Institute, Ministry of Agriculture. Five sexually mature healthy Holstein bulls with ranged of 3-4 years old age. These bulls were housed in individual boxes and fed on the recommended ration according to Animal Production Research Institute **(1975).** Semen was collected weekly using an artificial vagina for a period of 10 weeks from (April to June 2017). Immediately following the every collection, only ejaculates with low motile spermatozoa (estimated 45 to 50% motility) were pooled to eliminate the bull effect, then diluted with Tris extender to contain $80x10^6$ spermatozoa/ml (diluted rate 1:10) that had been previously warmed to 37 ºC and divided into five parts then subjected to gradually cooling from 37 ºC to 4 ºC for 4 h (as equilibration period) in a refrigerated unit before being placed into straws (0.25 ml French straws). The straws were held for 10 min at the surface of the liquid nitrogen vapor (~120°C) before being immersed then stored in liquid nitrogen (-196°C).

***Preparation of the extenders*:**

Tris-egg yolk extender (20% EY) containing 20 ml of egg yolk, 3.025g Tris ( hydroxyl methyl amino methane), 1.675 g citric acid, 0.75 g glucose, 7 ml glycerol, 0.25 gm lincomycin, 0.005 gm streptomycin and completed with up to 100 ml bidistilled water, this extender was acting as control. L-arginine was added to Tris basic extender (control) at the concentration of 0, 3, 5, 7 and 10 mM.

Semen characteristics of fresh semen collected from 5 bulls showed in Table (1). Semen was evaluated as volume, mass motility, live sperm, abnormal sperm and sperm concentration according to Salisbury *et al.* (1978).

***Semen processing:***

The percentage sperm progressive motility, live sperm, abnormal sperm and acrosome integrity were evaluated post dilution, post equilibration and post frozen-thawed spermatozoa. Progressive sperm motility was estimated according to Melrose and Laing (1970), while acrosome integrity estimated according to Watson (1975). Also DNA damage was performed post-thawing only according to Liu and Baker (1994).

**Table (1):** Semen characteristics (Mean ±S.E) of fresh semen collected from 5 bulls in the present experiment.

| Bulls | Volume (ml) | Mass motility (%) | Live spermatozoa (%) | Abnormal spermatozoa (%) | Concentration ($\times 10^9$/ml) |
|---|---|---|---|---|---|
| Bull1 | 3.3± 0.28 | 52.8±1.40 | 60.4±0.83 | 31.4±1.22 | 0.89±0.13 |
| Bull2 | 2.8± 0.24 | 54.3±1.23 | 63.9±0.91 | 29.0±1.20 | 1.20±0.07 |
| Bull3 | 3.0±0.35 | 60.5±1.61 | 69.7±0.87 | 25.3±1.10 | 1.42±0.15 |
| Bull4 | 2.8±0.30 | 55.4±1.31 | 65.6±0.78 | 30.1±1.01 | 1.34±0.20 |
| Bull5 | 2.9±0.27 | 57.6±1.04 | 66.4±0.71 | 26.4±1.13 | 0.93±0.09 |

***Acrosome Integrity:***

Acrosome integrity was determined by using a Giemsa stain procedure as described by Watson (1975). A drop of diluted semen was smeared on a pre-warmed slide and dried in a current of warm air. The smears were fixed by immersion in 10% buffered formal saline for 15 minutes, and then washed in running tape water. The smears were air dried and then immersed in buffered Giemsa solution (3 ml Giemsa stock solution was diluted with 2 ml of Sorensen's buffer, pH 7, and 35 ml distilled water) in a coplin jar for 90 minutes after which they were rinsed briefly in distilled water and dried.

The dried smears were studied under a light microscope at magnification of 100x using oil immersion lens. The percentage of normal acrosome was calculated for about 200 spermatozoa randomly selected from at least four microscopic fields. The acrosome was considered to be normal when the stain was clearly and evenly distributed over the spermatozoa anterior to equatorial segment.

***Chromatin integrity by Acridine Orange Test (AOT):***

Frozen semen was washed in 5 ml of PBS (phosphate buffered saline). After centrifugation, the sperm pellet was re-suspended in 0.5 ml of phosphate buffered saline. A small aliquot (50 µL) of the sperm suspension was then glass smeared. Three smears from each sample were prepared on glass slides, air dried and then fixed overnight in Carnoy's solution (methanol/acetic acid, 3:1) according to Liu and Baker (1994). Once rinsed and air dried, the slides were stained for 5 min with freshly prepared acridine orange (AO) stain as follows: 10 ml 1% AO in distilled water was added to a mixture of 40 ml 0.1 M citric acid and 2.5 ml 0.3 M $Na_2HPO_47H_2O$. The AO solution was stored in a dark place at 4°C for four weeks. After washing and drying, the slides were examined using a fluorescent microscope (Leitz, Germany; excitation of 450-

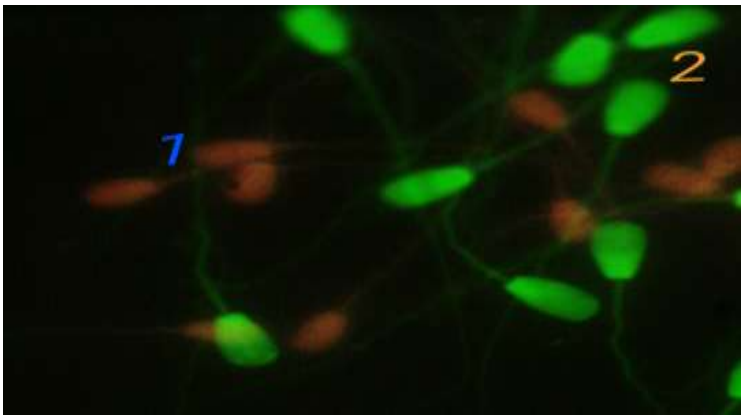


**Photo (1). Normal and abnormal DNA**
1-Abnormal DNA 2-Normal DNA

490 nm). Sperm with intact chromatin or normal DNA content had green fluorescence, whereas sperm with an abnormal DNA content emitted fluorescence in a spectrum varying from yellow-green to red.

***Fertility trial:***

A total of 120 Friesian cows owned by small and medium scale breeding holder in different villages in Kafr-Elsheikh Governorates were artificially inseminated with random frozen doses from various extenders. Each female was inseminated with a single straw 10 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:***

Data obtained were subjected to analysis of variance (ANOVA) one way using **SAS** (2001) after arcsine transformation and Duncan Multiple Range Test (Duncan, 1955) was used to separate significantly different means. The model that was used to analyze the data is stated below:

$$Yij= \mu + Ai + eij,$$

Where:

Yij= Dependent variables, μ= Population mean, Ai= Effect due to L-arginine (i = 0, 3, 5, 7 and 10 mM), eij= Experimental error.

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## RESULTS AND DISCUSSION

***The percentage of progressive sperm motility:***

Effect of L-arginine concentration on progressive motility of poor quality is presented on Table (2).

Statistical analysis revered that no significant (P<0.05) differences were detected among treated and control groups in progressive motility post-dilution. Treatments group (3, 5, 7 and 10Mm) were significantly higher (P<0.05) in the percentage progressive motility than in control group (44.5%) at post-equilibration period, however no significant differences among treatments G3, G4 and G5 and between G2 and G5. Data showed that the best results for progressive motility were recorded with G3 and G4 (54, 53.5%).

The percentage of progressive motility in all treated groups post-thawing was significantly higher as compared with control group. The percentage of progressive motility was increased in G5 with significant. Concerning sperm motility, the addition of L-arginine in 5 and 7mM concentrations enhances sperm motility following equilibration and freezing-thawing processes by increasing production of nitric oxide which enhances the metabolic rate, it also enhances cGMP synthesis, thus leading to increase the calcium level in the mitochondria and generating a higher ATP (Zini *et al*., 1995 and Revelli *et al*., 2001). Govil *et al.* (1992) reported that arginine protects spermatozoa against lipid peroxidation through increasing nitric oxide production. As mentioned before, nitric oxide is synthesized from arginine by a family of isoenzymes known as the nitric oxide syntheses (Pacher *et al.*, 2007) based on this, arginine has been known to increase generation of nitric oxide. Also, arginine acts as an antioxidant which protects spermatozoa against lipid peroxidation during storage through increasing nitric oxide production which reduces lipid peroxidation by inactivating free radicals.

**Table (2):** Effect of L-Arginine concentration in extender of moderate semen quality of Holstein bull on progressive motility (%) (Mean±S.E) at different stages of cryopreservation.

| Items | Treatment groups | | | | |
|---|---|---|---|---|---|
| | | L-Arginine | | | |
| | G1 | G2 | G3 | G4 | G5 |
| **Post-dilution** | 52.5 ±1.9 | 52.5±2.5 | 55.0±2.4 | 55.5±2.1 | 54.5±1.4 |
| **Post-equilibration** | $44.5^{C}$±1.5 | $50.0^{B}$±2.7 | $54.0^{A}$±2.1 | $53.5^{A}$±2.4 | $52.0^{AB}$±1.8 |
| **Post–thawing** | $23.5^{B}$±1.5 | $40.0^{A}$±1.8 | $44.8^{A}$±2.1 | $45.5^{A}$±2.0 | $41.5^{A}$±1.9 |

Means having. different letters superscripts within the same row was differ significant (P<0.05)

***The percentage of live spermatozoa:***

The percentage live spermatozoa are illustrated in Table 3. There is no significant (P<0.05) differences concentration of L-arginine on live spermatozoa compared with control at post-dilution stage, however at post-equilibration the significant (P<0.05) increase in percentage live spermatozoa (%) with increment with L-arginine concentrations 3,5,7 and 10mM (57.2, 61.0, 62.5 and 56.0%) corresponding to control (51.0%) in extender of low semen quality respectively. On the other hand, statistically significant (P<0.05) differences were observed among the concentrations of L-arginine on live sperm as follow (50.0, 56.8, 57.5, 52.5%) compared with control (39.5%) at post-thawing of cryopreservation. Concerning increasing livability and decreasing abnormal sperm percentages, L-arginine plays an important role as an antioxidant by inactivating superoxide anion due to increasing nitric oxide production thereby decreasing lipid peroxidation of sperm membrane. The present results were agreement with the findings of Al-Ebady *et al.* (2012) who recorded that L-arginine can be used to activate the motility of poor motile bull spermatozoa, furthermore, it can be used as an adjuvant in bull semen extenders to maintain the viability of spermatozoa after cryopreservation in liquid nitrogen at -196C°. On the other hand, Öztürk *et al.* (2017) found that supplementation of bovine semen extender with arginine decreased the percentages of post-thawed subjective motility. Hassanpour *et al.* (2010) found that low concentrations of L-arginine (0.001, 0.01 and 0.1mM) had little effect on sperm motion parameters; whereas high concentration of L-arginine significantly decreased specific motion parameters in ram epididymal sperm. Moreover, O'Flaherty *et al.* (2004) showed that viability of bovine spermatozoa remained unchanged with time and various concentrations of L-arginine (1.25 to 30 mM). They indicated that L-arginine does not have any adverse effect on viability of the cells throughout the course of the experiment.

**Table (3):** Effect of L-Arginine concentration in extender of poor semen quality of Holstein bull on live spermatozoa (%) (Mean±S.E) at different stages of cryopreservation.

| Items | Treatment groups | | | | |
|---|---|---|---|---|---|
| | G1 | L-Arginine | | | |
| | | G2 | G3 | G4 | G4 |
| **Post-dilution** | 64.7 ±1.1 | 63.5±1.4 | 67.0±1.2 | 66.3±1.5 | 65.2±1.3 |
| **Post-equilibration** | $51.0^{B}$±1.9 | $57.2^{AB}$±2.3 | $61.0^{A}$±1.8 | $62.5^{A}$±2.0 | $56.0^{AB}$±2.1 |
| **Post–thawing** | $39.5^{B}$±1.7 | $50.0^{AB}$±2.0 | $56.8^{A}$±2.1 | $57.5^{A}$±1.9 | $52.5^{AB}$±1.8 |

Means having. different letters superscripts within the same row was differ significant (P<0.05).

***The percentage of abnormal spermatozoa:***

Table 4 shows the effect of adding 3,5,7 and 10 mM of L-arginine to semen extender on percentage abnormalities of moderate motile bull sperms after (dilution, equilibration and thawing) period. The percentage of abnormalities were without significant (P<0.05) differences when compared with the control. After equilibration the percentage increased to (37.3%) in the control, where as it decreased in treated parts to (34.1, 30.1, 31.4 and 35.1%) with significant (P<0.05) differences when compared with control. However the percentage in treated parts after thawing decreased to (38.0, 34.4, 35.3 and 39.6 %) with significant (P<0.05) differences compared with the control (43.2%).

**Table (4):** Effect of L-Arginine concentration in extender of poor semen quality of Holstein bull on abnormal spermatozoa (%) (Mean±S.E) at different stages of cryopreservation.

| Items | Treatment groups | | | | |
|---|---|---|---|---|---|
| | G1 | L-Arginine | | | |
| | | G2 | G3 | G4 | G5 |
| **Post-dilution** | 29.6 ±0.9 | 27.2±0.5 | 26.6±0.6 | 28.7±0.7 | 30.4±0.8 |
| **Post-equilibration** | $37.3^{A}$±1.0 | $34.1^{AB}$±0.8 | $30.1^{B}$±1.1 | $31.4^{B}$±0.9 | $35.1^{AB}$±1.2 |
| **Post–thawing** | $43.2^{A}$±1.2 | $38.0^{B}$±1.0 | $34.4^{C}$±1.3 | $35.3^{C}$±1.1 | $39.6^{B}$±1.4 |

Means having. different letters superscripts within the same row was differ significant (P<0.05).

***The percentage of acrosome integrity:***

Data presented in Table 5 shows changes in values of acrosomal integrity of moderate motile sperms. The data revealed that adding different concentrations of L- arginine increase the percentage of acrosomal intact of sperms when compared with the control during the whole period of preservation, and best concentrations of L- arginine gave higher percentage of acrosomal integrity sperms in all periods of storage was (5 and 7 mM) with significant (P < 0.05) differences when compared with the control. In the present study, addition of 5 or 7 mM L-arginine to bull extenders increased (P < 0.05) significantly the percentage of acrosome integrity compared to the control and other 3 or 10 mM L-arginine extenders. On the other hand, Öztürk *et al*. (2017) found that the percentage of acrosome integrity did not varied significantly with addition of L-arginine to semen extender. Moreover, AL-Ebady *et al*. (2012) found no significant differences in acrosome integrity between control and treated part of semen with L-arginine after freezing.

**Table (5):** Effect of L-Arginine concentration in extender of poor semen quality of Holstein bull on acrosome integrity (%) (Mean±S.E) at different stages of cryopreservation..

| Items | Treatment groups | | | | |
|---|---|---|---|---|---|
| | | L-Arginine | | | |
| | G1 | G2 | G3 | G4 | G5 |
| **Post– dilution** | $60.2^{B}$±1.9 | $65.0^{AB}$±2.6 | $68.3^{A}$±2.5 | $69.4^{A}$±3.1 | $67.5^{A}$±1.5 |
| **Post-equilibration** | $51.3^{B}$±2.1 | $60.5^{A}$±2.8 | $65.3^{A}$±2.2 | $66.7^{A}$±2.6 | $65.9^{A}$±2.3 |
| **Post–thawing** | $39.2^{B}$±2.9 | $47.2^{B}$±4.5 | $57.2^{A}$±3.3 | $58.9^{A}$±3.2 | $50.9^{B}$±4.1 |

Means having. different letters superscripts within the same row was differ significant (P<0.05).

***Chromatin integrity by acridine orange test (AOT):***

The chromatin integrity of sperm was stained using acridine orange technique (Table 6).

The percentage of DNA damage were 20.0, 10.8, 4.40, 3.20 and 9.40 for control and frozen semen supplemented with L- arginine at concentrations 3,5,7 and 10 mM, respectively. Significant differences in chromatin integrity were observed among control and treatments (P<0. 05). There were no differences between the concentrations of L- arginine 3 and 10 mM for DNA damage and there were no differences between the concentrations of L-arginine 5 and 7mM.

**Table (6):** Percentage of cow sperm cells with DNA damaged detected by acridine orange test in semen (mean ± SE).

| Treatment groups | DNA damage (%) |
|---|---|
| G1 | $20.0^{A}$ ± 0.91 |
| G2 | $10.8^{B}$ ± 0.76 |
| G3 | $4.40^{C}$ ± 0.51 |
| G4 | $3.20^{C}$ ± 0.43 |
| G5 | $9.40^{B}$ ± 0.63 |

Means having. different letters superscripts within the same row was differ significant (P<0.05).

Sperm with normal intact chromatin or normal DNA had a green flourscence, whereas sperm with damaged chromatin or abnormal DNA content had a red flourscence. Green sperm indicated that the stain interact with native DNA and flourscence green, but the sperm with single stranded DNA or damaged chromatin turned flourscence red. Concerning the sperm chromatin integrity, the addition of different L-arginine concentrations (3, 5, 7 and 10 mM) with the semen extenders led to reduce (P<0.05) sperm DNA

damaged compared to the control extender. Sperm freezing increases the percentage of DNA fragmentation. This effect occurs after cryopreservation and has been reported by several authors (Thomson *et al*., 2009) like in our study, Ogretmen *et al*. (2015) also reported that extenders with antioxidant additives had protective effects against sperm DNA damage resulting from cryopreservation. Öztürk *et al*. (2017) found that using L-arginine with semen extender led to decrease damage DNA ($P<0.05$) compared to the control. Potts *et al*. (2000) has reported DNA damage in human spermatozoa connected with ROS and lipid peroxidation.

***Conception rate:***

In the study, overall conception rate using frozen semen 60.8% (73 conceptions from 120 inseminations). The conception rate of frozen poor motility semen treated with different concentrations of L- arginine (3,5,7 and 10 mM) was high significant difference ($P<0.05$) (60.0, 65.0, 66.0 and 63.3%) compared with control (45.0%) respectively. Concerning the conception rate, the using of frozen-thawed semen supplemented with different L-arginine levels for insemination led to increase ($P<0.05$) fertilized cows compared to control. In general, conception rate means the success rate of artificial insemination in agricultural animals, usually expressed as a percentage.

**Table (7):** Conception rate of Friesian - cows inseminated with frozen semen cryopreserved in different concentrations of L-Arginine.

| Treatment groups | No. of inseminated females | No. of conceived females | Conception rate (%) |
|---|---|---|---|
| **G1** | 20 | 9 | $45^{c}$ |
| **G2** | 25 | 15 | $60^{b}$ |
| **G3** | 20 | 13 | $65^{a}$ |
| **G4** | 25 | 17 | $66^{a}$ |
| **G5** | 30 | 19 | $63.3^{b}$ |

Means having. different letters superscripts within the same row was differ significant ($P<0.05$).

Any treatment applied to semen is likely to affect the fertilization rate. The probability that a spermatozoon will fertilize egg depends on at least one spermatozoon surviving at the site of fertilization until the ovum arrives, and this is dependent upon insemination of enough spermatozoa, the survival of spermatozoa in the female tract and the time of insemination in relation to ovulation. These three factors are not independent because deficiencies in one or two of them can be compensated for by excess in the others (Shanon P., 1968).

Based on conception rate (CR) results in this study are presented in Table (7). Reproduction efficiency could be stated as good if CR reaching 65–66% (Kaufmann *et al.,* (2009). The CR results in this study by using frozen of poor quality semen treated with 5mm and 7mm l- arginine was 65 and 66% respectively, which was much better compared to the research result reported by Fair *et al.,* (2004) with successful rate of 40–62% on AI method and using frozen semen this explained by addition of optimum concentrations of l-arginine (5 and 7mM) to poor quality semen lead to decrease the percentage of abnormal sperm, decrease damage DNA, increase the progressive motility and the acrosome integrity of poor motility spermatozoa.

## CONCLUSION

In the present study, under our experimental conditions treatment of poor bovine spermatozoa with L-arginine at concentration of 5 mM and 7mM was considered the best concentrations to be used to decrease the percentage of abnormal sperm, decrease damage DNA, increase the progressive motility and the acrosome integrity of poor motility spermatozoa and conception rate. More detailed studies are required to confirm the benefits of using L-arginine in extender of poor quality semen.

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## تأثير اضافة ل - ارجنين لمخفف السائل المنوى منخفض الحيوية لطلائق الهولشتين

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أجريت هذه الدراسة لمعرفة تأثير مستويات مختلفة من L- أرجينين (٠ ، ٣ ، ٥ ، ٧ و ١٠ ملى مول) على خصائص السائل المنوي منخفض الحيوية لطلائق الهولشتين

. تم جمع السائل المنوى من خمسة طلائق هولشتين أسبوعيا ذو حركة كلية ٥٠-٦٠ ٪
. تم تخفيف السائل المنوي عند ٣٧ درجة مئوية باستخدام مخفف الترس-صفار البيض وتم تقسيمه إلى خمسة أجزاء ، الجزء الأول بدون اضافة (كنترول) ، وتم استكمال الأجزاء الأخرى ٢ ، ٣ ، ٤ و ٥ بـ ٣ ، ٥ ، ٧ و ١٠ ملي مول على التوالى. تم وضع السائل المنوي المخفف على ٤ درجة مئوية لمدة ٤ ساعات كفترة توازن، تم تعبئة السائل المنوى المخفف في قصيبات سعة ٠.٢٥ مل ، وتم وضعها على أبخرة النيتروجين لمدة ١٠ دقائق ثم تغمس فى النتروجين السائل (-١٩٦ درجة مئوية). تم تقدير(الحركة التقدمية ، الحيوانات المنوية الحية ، الحيوانات المنوية غير الطبيعية وسلامة الأكروسوم) أثناء مراحل الحفظ المختلفة.

وقد أشيرت النتائج إلى أن المخفف ذو التركيز ٥ و ٧ ملى مول L - أرجينين كانت فعالة في الحركة الفردية ، الحيوانات المنوية الحية ، الحيوانات المنوية غير طبيعية وسلامة الأكروسوم و سلامة الكروماتين عن مخفف ٣ و ١٠ ملى مول L-أرجينين مقارنة مع الكنترول في مراحل التجميد المختلفة. أيضا ، كان معدل الخصوبة أعلى في الأبقار الفريزيان الملقحه اصطناعيا بتركيز ٥ و ٧ مل مول أرجينين (٦٥ و ٦٦ ٪) مقارنة مع الكنترول (٤٥ ٪).

**التوصية:** نستنتج من الدراسة انه يمكن اضافة الارجنين بتركيز ٥ أو ٧ ملى مول إلى المخفف والتى أدى إلى تحسين خواص السائل المنوى ضعيف الحركة و قابلية التجمد وزيادة نسبة الخصوبة .