# Effect of adding L.arginine on some parameters of bull sperms after freezing in liquid nitrogen (-196˚C).

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## Abstract

In order to investigate the effect of adding L.arginine on poor motile bull sperms, this study was conducted in Artificial Insemination Center of Abou-Ghareeb . 17 ejaculates with poor motile sperms estimated (40% to 55%) were collected by artificial vagina from 5 bulls and extended with Tris-yolk-fructose-glycerol extender supplemented with 0.005 M of L.arginine, cooled for 2 hours, equilibrated for 4 hours and frozen in liquid nitrogen at (-196˚C), then thawed after 48 hours in (37˚C for 30 seconds) . Determination for spermatozoal motility, dead and abnormalities percentages and acrosomal abnormalities percentage were evaluated before and after freezing. Results obtained in this study concluded that L.arginine can be used to activate the motility of poor motile bull sperms, furthermore, it can be used as an adjuvant in bull semen extenders to maintain the viability of spermatozoa after cryopreservation in liquid nitrogen at (-196˚C).

## Introduction

Artificial Insemination (A.I) has the ability to promote quick and broad improvement in genetic quality of cattle, because this technique enables superior genes to be spread widely amongst the cattle population. A.I is also useful to combat venereal diseases [1], and because of the simplicity of preserving and transporting the semen in this technique, A.I remains the most practical method for genetic upgrading of cattle in spite of major advances in other reproductive technologies in recent years [2]. Successful A.I depends on successful media 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 [3], therefore, various additives have been incorporated into semen extenders to improve sperm motility, longevity and thereby fertility [4]. L.arginine is amino acid plays an important role in stimulating sperm motility in rabbits [5], humans [6] and goats [7] under in vitro conditions. L.arginine produces nitric oxide by the action of nitric oxide syntheses enzyme. Nitric oxide is a molecule of great biological significance and has long been considered to play an important role in sperm physiology such as sperm motility, sperm-egg interaction and spermatogenesis[8], more over, nitric oxide participate in defense mechanism against reactive oxygen species formation during spermatozoa preservation under freezing in liquid nitrogen at (-196˚C), which lead to maintain post-thaw sperm motility and viability [9,10]. A.I center in Abou-Ghareeb is responsible for production of frozen semen in Iraq, and the center depends on the imported bulls that produces semen with high quality, but for known or unknown reasons, some bulls produce ejaculates with low quality, specially in sperm motility, and for this reason these poor ejaculates were excluded, which lead to economical loss,

therefore, this study was conducted to know the effect of adding L.arginine to Tris-yolk-fructose-glycerol extender on spermatozoal motility, viability and longevity of poor motile bull sperms during cryopreservation in liquid nitrogen at (-196˚C), which may lead to open the door for using L.arginine as an adjuvant in semen extenders, in order to protect spermatozoa during preservation at freezing.

## Materials& Methods

This study was carried out at artificial insemination center of Abou-Ghareeb western of Baghdad, on (5) Holstein bulls imported from Australia. Semen was routinely collected from all bulls weekly with the aid of an artificial vagina. All bulls have the same age (4-5 years), and were kept under identical conditions of management, feeding and watering throughout the study period. Poor motile ejaculates (estimated 40% to55% of individual motility) were taken weekly. These ejaculates were excluded from semen-freeze processing in this center due to poor motility. Total of 17 ejaculates were studied. As a routine work in the A.I center, after collection of semen, the sample was immediately brought to the laboratory, and placed in a water bath at (37-38˚C) for evaluation of volume, mass activity [11], individual motility [12], and sperm concentration [13]. Only poor motile ejaculates were chosen for this study. Ejaculates were diluted with Tris-yolk-fructose-glycerol (1:10 diluted rate). The diluted semen was divided into two equal parts in two plastic test tubes, one used as control without L.arginine, and the other was supplemented with L.arginine 0.005 molar/ml. The two test tubes were placed in a beaker containing warm water about (30-32˚C) and the beaker was sited at room temperature for 30 minutes, which is the optimum time for maximum L.arginine intake [5]. After 30 minutes, the diluted semen in the treated and the control test tubes was evaluated for motility, dead and abnormalities percentages [2] and acrosomal abnormalities percentage [14]. After 30 minutes at room temperature, the beaker which contained the two test tubes was transferred into the cold cabinet and cooled for 5˚C for 2 hours, then equilibrated for 4 hours. During the equilibration time, the diluted semen evaluated for motility, dead and abnormalities percentages and acrosomal abnormalities percentage, then the diluted semen packaged in straws 0.25ml and freezed in liquid nitrogen, and stored according to the freezing process as a routine work in the A.I center. After 48 hours of storage, thawing is achieved by placing the straw into a water bath at 37˚C for 30 seconds, then the semen evaluated for the percentages of motility, dead , abnormalities, and acrosomal abnormalities. The data from this study were analyzed by statistical analysis system [15].

## Results &Discussion

The table shows the effect of adding 0.005 molar/ml of L.arginine on some physical properties of poor motile bull sperms before and after freezing in liquid nitrogen at (-196˚C). As depicted in the table, the individual motility was (44.41%) in control, whereas the motility in treated part increased significantly ($P<0.05$) to (62.35%) after 30 minutes of adding (0.005 molar) of L.arginine . The motility in control after 2 hours of cooling to 5˚C decreased to (40.29%) , whereas in treated part the motility decreased to (59.41%)with significant ($P<0.05$) differences when compared with control. After freezing for 48 hours and thawing in 37˚C for 30 seconds, the motility in control decreased to (24.12%) , whereas in treated part decreased to (46.76%) with significant ($P<0.05$) differences when compared with the control. Concerning dead percentage, it was (18.53%) in control before cooling whereas the percentage in treated part

was (18.56%) with no significant (P<0.05) differences when compared with control. After 2 hours of cooling to 5˚C, the percentage increased to (25.03%) in control, whereas the percentage in treated part increased to (20.38%) with significant (P<0.05) differences when compared with the control. Dead percentage in control after freezing and thawing increased to (62.1%), whereas the percentage in treated part increased to (36.23%) with significant (P<0.05) differences when compared with the control. Concerning abnormalities, the percentage in control before cooling was (7.08%), whereas the percentage in treated part was (7.12%) without significant (P<0.05) differences when compared with control. After 2 hours of cooling to 5˚C, the percentage of abnormalities in control increased to (7.97%) whereas in treated part the percentage increased to (7.7%) without significant (P<0.05) differences when compared with the control. After freezing and thawing the percentage increased to (10.76%) in the control, whereas it increased in treated part to (10.03%) without significant (P<0.05) differences when compared with control. Concerning acrosomal abnormalities, the percentage in control was (3.17%) in both control and treated part of semen before cooling, and increased to (3.23%) in both parts after 2 hours of cooling to 5˚C. The percentage of acrosomal abnormalities in control after freezing in liquid nitrogen increased to (7.14%) whereas the percentage in treated part after freezing increased to (6.94%) without significant (P<0.05) differences compared with the control.Results in the table showed that adding (0.005 molar) of L.arginine to poor motile bull sperms increased significantly (P<0.05) the percentage of motile sperms when compared with the control before and after freezing, and decreased significantly (P<0.05) the percentage of dead sperms when compared with the control after freezing, whereas the percentage of sperm abnormalities and acrosomal abnormalities decreased after freezing but without significant (P<0.05) differences when compared with the control. Concerning sperm motility, the addition of L.arginine in low concentrations enhances sperm motility 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 level. These two effects lead to increase sperm motility [16 – 19]. Concerning decreasing dead and abnormalities 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 lipids of sperm membrane (which are mainly phospholipids) are highly susceptible to the action of peroxidizing agents, which may be natural or present due to extraneous factors [20]. During preservation, spermatozoa exposed to radiation, cold shock or various preservatives, and such exposure may lead to peroxidation which forms lipid peroxides. The peroxides constitute a potential hazard to the structural and functional integrity of spermatozoa, which lessening motility and metabolic activity of cells during storage in vitro [21,22]. Exogenous nitric oxide released by adding nitric oxide donors such as sodium nitroprusside which known to play an important role in sperm hyper activation in vitro and is beneficial for the maintenance of post-thaw sperm motility and viability [9,23,24]. The results of this study are similar to results obtained by [15] when they used L.arginine as an antioxidant in semen extender to improve post-thaw goat sperms motility and viability. Concerning no significant (P<0.05) differences in acrosomal and sperm abnormalities between control and treated part of semen with L.arginine after freezing, this might refer to individual variations between bulls during freezing. It has long

been recognized that the individual bulls producing the semen are the major source of variation in all investigations of frozen semen [25]. There were male to male variability in sensitivity of spermatozoa to cryopreservation, and this individual variation among bulls have the effect on cryprotective properties and spermatozoa viability post-thaw [26 – 29].

Table (1).The effect of adding 0.005 M of L. arginine on some semen characteristics before and after freezing

| The stage | Treatment | Individual motility | Dead Sperm (%) | Abnormal Sperm (%) | Abnormal acrosome (%) |
|---|---|---|---|---|---|
| before cooling | Control | A 44.41± 0.844 | A 18.53± 0.644 | A 7.08± 0.301 | A 3.17± 0.181 |
| | Treated | B 62.35± 1.492 | A 18.56± 0.649 | A 7.12± 0.297 | A 3.17± 0.181 |
| after cooling | Control | A 40.29± 0.906 | A 25.03± 0.878 | A 7.97± 0.319 | A 3.23± 0.171 |
| | Treated | B 59.41± 1.477 | B 20.38± 0.666 | A 7.70± 0.287 | A 3.23± 0.171 |
| After freezing | Control | A 24.12± 0.980 | A 62.10± 1.517 | A 10.76± 0.459 | A 7.14± 0.373 |
| | Treated | B 46.76± 1.129 | B 36.23± 1.208 | A 10.03± 0.359 | A 6.94± 0.353 |

Values are Mean ± SE.
Letters differences ($P<0.05$) between control and treated

The conclusion of this study, is that the extension of bull semen with Tris- yolk- fructose extender supplemented with L.arginine at a concentration of (0.005) molar / ml can be used as an agent to improve the motility of poor motile bull sperms and to improve post-thaw sperm motility and viability after cryopreservation in liquid nitrogen at (-196˚C).

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# تأثير إضافة الأرجنين على بعض معايير نطف الثيران بعد التجميد بالنيتروجين السائل بدرجة (−196°م).

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**الخلاصة**

لغرض دراسة تأثير إضافة الأرجنين إلى السائل المنوي للثيران الحاوي على نطف ضعيفة الحركة، أجريت هذه الدراسة في مركز التلقيح الاصطناعي في أبوغريب.17 قذفة منوية حاوية على نطف ضعيفة الحركة قدرت الحركة فيها 40٪ إلى 55٪، جمعت بواسطة المهبل الإصطناعي من 5 ثيران، وتم تخفيفها بمخفف الترس− صفارالبيض−الفركتوز−الكليسيرول المضاف إليه 0,005 مولار/ مل من الأرجنين، ومن ثم بردت لمدة ساعتين وعودلت لمدة أربع ساعات وجمدت بالنيتروجين السائل بدرجة −196°م، وبعد ذلك أجري إذابتها بعد 48 ساعة بدرجة حرارة 37 مئوية لمدة 30 ثانية. تم تقييم النسبة المئوية لحركة النطف الفردية ونسبتي النطف الميتة والمشوهة ونسبة تشوهات الأكروسوم قبل وبعد التجميد.النتائج التي تم التوصل إليها من خلال هذه التجربة أفضت إلى أن الأرجنين يمكن إستعماله لتنشيط حركة النطف الضعيفة للثيران، إضافة إلى أنه يمكن إستخدامه في مخففات مني الثيران كمادة مساعدة للمحافظة على حيوية النطف بعد حفظها بالنيتروجين السائل بدرجة −196 م.