Re-Dilution Effects and Arginine Addition for Activation Poor Post-Thaw Motility of Sperms for Holstein Bulls Born in Iraq

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Abstract

This study was carried out in Artificial Insemination Centre in Iraq to study the effects of specific additions Arginine in , re-dilution as activators to poor post thaw sperms motility of Holstein bull semen, as well as to recorded the effect of addition's time through 0,1 and 2hrs.Ahandered seventy two ejaculates of poor individual motility(40-45%) after freezing were collected from six Holstein bulls and frozen(- 196 C º) in straws were thawed in 37C° water bath for 30 minutes. Each collected samples were set in four test tubes in the water bath 37C° according to the addition used: control tube, re-dilution with 1ml Tris, 0.006 molar/ml of Arginine and 0.006 molar/ml of Arginine +1ml of Tris. The properties of sperm motility, dead, abnormalities and intact of acrosoma percentage were evaluated through the time 0, 1 and 2 hr of setting the samples in water-bath. Results had showed that adding Arginine (with or without Tris diluents) increases significant ($P < 0.05$) the individual motility and acrosoma intact during the storage period 1 and 2hour comparison with control and Tris without Arginine , whereas percentage of dead and abnormality of these poor post-thaw motility decrease significantly ($P < 0.05$) in samples which were contain Arginine (with or without Tris), during storage period 1and 2 hour, while the motility , dead , abnormality and intact of acrosoma for poor sperms don't different significantly between all treatments during the storage period. Moreover these parameters were didn't differences significant when comparison between control with Tris, and Arginine with Arginine + Tris. It is concluded that after 1 hour of adding Arginine could used to activate the poor post-thaw motility of sperms, and could used as an adjuvant in post-thaw bull semen to maintain the viability of spermatozoa after preservation 1 or 2 hours at 37°C water-bath, but directly (0hr) adding Aginine does not increase live ability of poor post-thaw bulls sperms.

KEYWORDS: Re-dilution, Arginine, activation, poor motility, Holstein bulls, Iraq.

INTRODUCTION

Artificial insemination (AI) has contributed enormously to the genetic improvement of beef and dairy cattle in the last 50 years. Geographical barriers to breeding animals have long been reduced because of possibilities of semen transportation (Alemayehu , 2011).Frozen semen in straws has become the universally accepted unit of storage and transfer of bovine genetics to cattle producers. Freezing and thawing of bull semen leads to a decrease in percentage of intact sperm, reducing the percentage of viable sperm cells to approximately 50–60% (Woeleder et al 1997). Longer-term storage of semen is achieved through cryopreservation. 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) . Freezability is the ability of spermatozoa to tolerate against freezing. The freezability

of spermatozoa ranges according to individual, and in the same bull it ranges according to the conditions of semen collection, its age and season when the semen collection is carried out (Yamashita, 2004). Various additives have been incorporated into semen extenders to improve sperm motility, longevity and there by fertility (Maule 1962). 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 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(Revelli et al 2002), moreover, nitric oxide participate in defense mechanism against reactive oxygen species formation during spermatozoa preservation under freezing in liquid nitrogen at (-196C˚), which lead to maintain post-thaw sperm motility and viability (Hellstrom et al 1994 , Srivastava et al 2006). AI center in Abo Ghareeb is responsible for processing of frozen semen in Iraq .Ejaculates with low freezability ejaculates (sperms motility 40-45%) , these poor ejaculates excluded from processing in AI center, which lead to economical loss, therefore, this study was conducted as attempt to:

1- Reducing cost manufacture of frozen this discharged ejaculates by re-dilution with Tris and activation by Arginine, it means used as liquid semen during insemination for semen package in straws but this technique can be used normally for activation poor post-thaw motility were package in ampoules or pellets.
2- Know the livability and longevity of poor post-thaw motility of sperms after re-dilution and addition Arginine.

MATERIALS AND METHODS

This study is carried out in artificial insemination (AI) centre in Abo Ghreeb, on six Holstein bulls born in Iraq. Semen was routinely collected from all bulls weekly with the aid of an artificial vagina. All bulls have the same age 29-32 months and were kept under identical conditions of management, feeding, and watering throughout the study period. All ejaculate were immediately brought to the laboratory, and placed in a water bath at 37C° for evaluation volume of semen, mass activity, individual motility (Chemineau, et al,1991), after that semen diluted with Tris- yolk- glycerol diluter (1:8-10 folds), and transferred beaker contain semen diluents to the cold cabinet, in a controlled manner, allow to reach the stabilize degree 5C° in about 1-1.5 hours, the diluted semen is held at this temperature (5C°) for 4 hours equilibration time, which calculated when the degree of water in the beaker reached 5°C,and during this time diluted semen packaging in straws 0.25ml automatically, after equilibration the freezing in liquid nitrogen vapour (-120C°) and storage in liquid nitrogen (-196C°) for 48hr, after that and as a routine work in AI centre one straws from each ejaculates thawing into water bath at 37C° for 30 sec to selection straws give individual motility 50% or more and discharge straws with motility less 50%.So this study concentrated on 172 straws (post-thaw poor motility 40-45%) and each four straws empty in 4 test tubes (first used as control, second, contain 1ml diluents (Tris-Fructose-egg yolk), third contain 0.006 M/ml Arginine and fourth contain 1ml above diluents and 0.006 M/ml Arginine, after incubation period of 0hr,1hr and 2 hr in water bath at 37C°, the semen in all treated and control test tubes was evaluated for individual motility

(Chemineau, et al,1991), dead and abnormalities percentage (Bearden, et al 2004) and intact of acrosomal percentage, it was calculated by using haematoxylin-eosin stain according to (WHO-NAFA, 2000) .

Statistical Analysis: Data were statically analysis (ANOVA) according to (SAS, 2000) using Duncan test for comparing between means under probability level of ($P< 0.05$).

RESULTS

Table (1) shows the effect of re-dilution and adding 0.006 molar/Ml of L.Arginine on some semen quality of poor motile bull sperms after freezing in liquid nitrogen at (-196 C˚). As depicted in this table,

1-individual motility of sperms: Table (1) display that using 0.006M/ml Arginine for re-dilution poor motile bull sperm cause increased ($P <0.05$) significantly sperm motility percentage after incubation period 1hr (54.66) and 2hr (56.78) , in addition to adding 0.006M/ml Arginine + Tris also increased ($P <0.05$) significantly sperm motility percentage after incubation period 1hr (56.30) and 2hr (58.23) ,when compared with the control and addition Tris in incubation period,1hr (36.34,38.87) and 2hr (30.05,31.06) , respectively. But during incubation period 0hr no difference was recorded between control with all treated parameters, and also during 1 and 2hr no significant differences between control with Tris and between 0.006M/ml Arginine with 0.006M/ml Arginine + Tris. Concerning study effect of incubation period (0,1 and 2hr) on individual motility for each treatment(Table 1) , also result revealed that sperm motility during incubation period 1hr for treated 0.006M/ml Arginine and 0.006M/ml Arginine + Tris (54.66,56.30) and 2hr (56.78,58.23) , respectively increased($P <0.05$) significant, comparative with incubation period 0hr(44.20,45.01) , respectively, but sperm motility during incubation period 2hr decreased($P <0.05$) significant for, treated Tris (31.06) compare with incubation period 0hr and 1hr (, 40.32, 38.87), respectively in addition to sperm motility during incubation period 2hr decreased($P <0.05$) significant for control (30.05) compare with incubation period 0hr (38.21) .

2-Dead of sperms : Table (1) conducted that using 0.006M/ml Arginine for re-dilution poor motile bull sperm cause decreased ($P <0.05$) significantly of dead sperms percentage

Table (1) Effect of Re-dilution and addition Arginine on semen characteristics of poor motility for bull sperms after freezing in liquid nitrogen (Means ±SE).

Semen characteristics	incubation period in 37°C	Control	Tris Diluent	Arginine	Arginine + Tris Diluent
Individual motility (%)	0hr	38.21 ± 5.65	40.32±6.01	44.2 ± 3.25	45.01±4.20
		Aa	Aa	Ba	Ba
	1hr	36.34 ± 4.32	38.87±4.31	54.66±6.45	56.30±4.32
		ABb	Ab	Aa	Aa
	2hr	30.05±2.66	31.06±5.19	56.78±6.41	58.23±3.89
		Bb	Bb	Aa	Aa
	0hr	47.16±3.45	45.56±2.34	40.02±4.31	41.43±3.56

(%)		Ba	Ba	Aa	Aa
	1hr	52.21±2.34	54.48±3.30	32.11±4.21	30.65±3.54
		Ba	Aa	Bb	Bb
	2hr	60.29±4.33	57.29±2.34	28.78±3.65	26.11±1.89
		Aa	Aa	Bb	Bb
Abnormalities sperms (%)	0hr	31.31±4.07	30.61±3.00	29.02±3.64	28.21±2.67
		Ba	Ba	Aa	Aa
	1hr	35.73±3.43	33.79±2.54	22.10±4.66	20.98±3.45
		ABa	ABa	Bb	Bb
	2hr	39.12±4.23	36.08±2.66	23.70±2.56	21.97±2.00
		Aa	Aa	Bb	Bb
Intact acrosoma (%)	0hr	43.33±3.11	45.89±2.96	40.42±4.31	39.43±2.06
		Aa	Aa	Ba	Ba
	1hr	28.42±4.14	30.98±3.77	50.13±4.00	53.05±3.94
		Bb	Bb	Aa	Aa
	2hr	25.88±4.23	30.19±4.34	47.78±3.65	48.11±3.81
		Bb	Bb	Aa	Aa

Differences small letters in same Row means different in significant ($P < 0.05$)

Differences Large letters in same Colum means different in significant ($P < 0.05$) after incubation period 1hr (32.11) and 2hr(28.78) ,in addition to adding 0.006M/ml Arginine + Tris also decreased significantly($P < 0.05$) dead of sperms percentage after incubation period 1hr (30.65) and 2hr (26.11) ,when compare with the control and addition diluents in incubation period,1hr (52.21, 54.48) and 2hr(60.29, 57.29) , respectively. But during incubation period 0hr no significantly differences between control as compare with all treated parameters, and also during 1 and 2hr no significant differences between control with Tris and between 0.006M/ml Arginine with 0.006M/ml Arginine + Tris. Concerning study effect of incubation period (0,1 and 2hr) on dead of sperms for each treatment Table (1) revealed that dead of sperms for treated 0.006M/ml Arginine and treated 0.006M/ml Arginine + Tris during incubation period 1(32.11,30.65) and 2hr (28.78,26.11) respectively, decreased ($P < 0.05$) significant compare with incubation period 0hr for treated 0.006M/ml Arginine and treated 0.006M/ml Arginine + Tris (40.02, 41.43) respectively, while percentage of dead sperms for treated Tris during incubation period 1hr (54.48) and 2hr (57.29) increased($P < 0.05$) significant compare with incubation period 0hr(45.56), in addition to dead of sperms for control during incubation period 2 hr(60.29) decreased ($P < 0.05$) significant compare with incubation period 0hr (47.16) and 1hr(52.21), on the other hand no significant differences between incubation period 0hr compare with 1hr for control and between incubation period 1hr compare with 2hr for treated Tris, 0.006M/ml Arginine and 0.006 M/ml Arginine + Tris (Table 1).

3-Abnormality of sperms: Table (1) observed that using 0.006M/ml Arginine and 0.006M/ml Arginine+ Tris for re-dilution poor motile bull sperm causes ($P < 0.05$) significantly decreased in abnormality of sperms during incubation period 1hr (22.10, 20.98) and 2hr(23.70, 21.97) compare with the control and treated Tris during incubation

period 1hr (35.75, 33.79) and 2hr (39.12,36.08) respectively . During incubation period 0hr no significantly differences between all treated parameters ,in addition to during incubation period 1hr and 2hr no significantly differences between control and treated Tris also between treated 0.006M/ml Arginine compare with treated 0.006M/ml Arginine+ Tris. Concerning study effect of incubation period 0,1 and 2hr on abnormalities for each treatment Table (1) revealed that abnormalities sperms for treated 0.006M/ml Arginine and 0.006M/ml Arginine + Tris during incubation period 1 (22.10, 20.98) and 2hr (23.70,21.97) , respectively were decreased (P <0.05) significant compare with incubation period 0hr(29.02) for0.006M/ml Arginine and (28.21) for and 0.006M/ml Arginine + Tris , but abnormality of sperms for treated Tris during incubation period 2hr(36.08) increased significant (P <0.05) compare with incubation period 0hr(30.61), in addition to abnormality of sperms for control during incubation period 2hr (39.12) increased significant (P <0.05) compare with period 0hr (31.31), but no significant differences between incubation period 0hr compared with 1hr for control and treated Tris and between incubation period 1hr comparison with 2hr for control and all treated (Table 1).

4-Intact acrosoma of sperms: Results Table (1) shows that using 0.006M/ml Arginine and 0.006M/ml Arginine + Tris for re-dilution poor motile bull sperm causes increased (P <0.05) significantly intact acrosoma of sperms when recorded during incubation period 1hr (50.13,53.05) compare with control (28.42) and treated Tris (30.98) , Table (1) also show, that during incubation period 2hr treated 0.006M/ml Arginine (47.78) and 0.006M/ml Arginine + Tris (48.11) increased acrosoma intact percentage significantly (P <0.05) compare with control (25.88) and treated Tris (30.19) , but during incubation period 0hr no differences significantly between all treated parameters and also during incubation period 1hr and 2 no differences significantly between control comparison with treated Tris hr and between treated 0.006M/ml Arginine compared with 0.006M/ml Arginine + Tris (Table1) . The effect of incubation period (0,1 and 2hr) on intact acrosoma of sperms for each treatment (Table 1),revealed that intact acrosoma of sperms for 0.006M/ml Arginine and treated 0.006M/ml Arginine + Tris during incubation period 1hr (50.13,53.05) and 2hr (47.78,48.11) increased (P <0.05) significantly compare with 0hr (40.42,39.43), respectively.Acrosoma intact of sperms for control and treated Tris during incubation period 1hr (28.42, 30.98) and 2hr (25.88,30.19) decreased (P <0.05)significantly compare with 0hr (43.33,45.89) respectively.

5-Effect of incubation period on percentage of individual motility, dead, abnormality and acrosoma intact of sperms). Results Table 1 conducted that the incubation period 2hr showed significant (P<0.05) gradual decrease of sperm motility and intact acrosoma of post -thaw sperm for control and treated Tris comparative with 0hr and between sperm motility during period 2hr comparison with 1hr for treated Tris .The results showed that the incubation period 2hr showed significant (P<0.05) gradual increase of dead and abnormalities sperms percentage of post –thaw for control and treated Tris compared with 0hr and between dead sperms during period 2hr comparison with 1hr for control (Table 1),in defiance of treated 0.006M/ml Arginine and 0.006 M/ml Arginine + Tris showed significant (P<0.05) gradual increase percentage of sperm motility and intact acrosoma but decrease percentage of dead and abnormality of post -thaw sperms when increase incubation period 1hr and 2hr (Table 1).

DISCUSSION

Results (Table 1) showing that re-dilution with Arginine of post-thaw semen enhanced quality of poor motility. Macmillan,et al (1968) reported that re-dilution of frozen semen caused increase in motility and livability of sperm. Srivastava, et al (2006) indicated that Arginine level has appositive correlate with sperm motility. AL-shaty in (2007) reaveled that the ability of L-Arginine to increase sperm motility in vitro raises the possibility of its potential clinical usefulness when semen with subnormal motility. Concerning sperm motility, as discussed before that addition of L.arginine in low concentrations enhances sperm motility by increasing production of nitric oxide which enhances cGMP synthesis, thus leading to enhancement of the metabolic rate (Chatterjee,et al ,2001,Wink et al 2001, Herrero et al 2003,Marquez and Suarez. 2004,). It also enhances the calcium level in the mitochondria and generating a higher ATP level, These two effects lead to increase sperm motility (Zini *et al*., 1995; Herrero *et al*., 1997; Sengoku *et al*., 1998 and Revilli *et al*.,2002,Ho etal,2002, Ho and Suarez ,2003, Jimenez-Gonzalez et al 2006). Results of this study indicated that addition of Arginine to post-thaw semen gives lower percentage of dead and abnormality of sperm. 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 (Belen Herrero et al 2000, Brouwers and Gadella, 2003). 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 (Jones *et al*., 1979). During preservation, spermatozoa exposed to radiation, cold shock or various preservatives, and such exposure may lead to peroxidation. 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 (Mann and Lutwak-mann, 1975 and Mann and Lutwak-mann, 1981). Also study revealed that addition Arginine maintenance of post-thaw sperm motility reflection control and addition Tris. Exogenous nitric oxide released by adding nitric oxide donors such as sodium nitroprusside which known to play an important role in sperm hyperactivation in vitro and it is beneficial for the maintenance of post-thaw sperm motility and viability (Sliwa and Stochmal, 2000). Result of effect incubation period on individual motility, dead, abnormality and acrosoma intact of sperm shows that incubation period 0hr of addition arginine no significant differences between all treated, Radany et al.,(1981) explain that 30 minutes which is the optimum time for maximum L.arginine intake ,so during incubation period 1 and 2hr control and treated Tris caused deterioter in quality of post-thaw semen but 0.006 M/ml Arginine and 0.006 M/ml Arginin + diluents enhanced this quality may be attribute to the positive effect which causing by addition Arginine , also results this study agreement with Al-Ebady (2012) which showing that adding Arginine to diluent semen increase livability of sperm which it is kept at 5°C for 120 hours. Exogenous nitric oxide released by adding nitric oxide donors such as sodium nitroprusside which known to play an important role in sperm hyperactivation in vitro and is beneficial for the maintenance of post-thaw sperm motility and viability (Hellstrom *et al*.,1994 ; Sharma and Agarwal.,1997 and Sliwa and stochmal,2000), on the other hand L.arginine has a positively churches that given an impression buffering effect according to dual reaction side that made the media neutralized partially (Hill and Briden,1999.

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