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Full Length Research Paper

Effect of adding arginine in different concentrations on some physical properties of poor motile bull sperms during different months

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In order to investigate the effect of adding arginine on poor motile bull sperms, this study was conducted in Artificial Insemination Center, Iraq, from December, 2008 to July, 2009. 114 ejaculates with poor motile sperms estimated (30 to 55%) were collected by artificial vagina (AV) from 10 Holstein bulls and extended with Tris-yolk-fructose extender supplemented with different concentrations of arginine (0, 0.002, 0.003, 0.004, 0.005 and 0.006 M/ml) for the determination of spermatozoa motility, dead and abnormalities percentages, and acrosoma abnormalities percentage. Results showed that adding different concentrations of arginine to poor motile bull sperms extended with Tris-yolk-fructose extender gradually increased the motility of spermatozoa but the best concentrations was 0.005 and 0.006 M/ml with significant ($P < 0.05$) differences when compared with the control during all month of this study, and months December and January gave better significant ($P < 0.05$) quality of semen compared with April, May, June and July, and last two months (June and July) gave lower significant ($P < 0.05$) different motility and high significant ($P < 0.05$) different percentage of dead, abnormality and abnormal acrosoma when compared with other months.

Key words: Arginine, physical properties, poor motile, bull sperm.

INTRODUCTION

Arginine takes part in sperm formation and has been found to be a basic component of the nucleoprotein of spermatozoa of various species (Adnan, 1970). There is correlation between arginine deficiency and loss of spermatogenesis and decrease in the motility of the sperm cell (Polakoski et al., 1976; Jungling and Bunge, 1976). Arginine plays a vital role in the maintenance of sperm motility and their metabolic activity inside reproductive tract or throughout storage *in vitro* conditions (Mann and Lutwak-mann, 1981). Also, arginine prevents bilayer phospholipids membrane peroxidation under various peroxidation situations through production of nitric oxide (NO) mechanism which protects structural and functional integrity of spermatozoa (Govil et al., 1992; Srivastava et al., 2000). Arginine promotes the sperm motility by improving the rate of glycolysis which elevates the rate of Adenosine-5'-triphosphate (ATP) and lactate generation in spermatozoa (Patel et al., 1998).

NO promotes mouse sperm capacitation and acrosoma reaction (Griveau et al., 1995). Several researches indicated that NO generated by human spermatozoa regulates sperm capacitation and associated protein tyrosine phosphorylation that mediated through a cAMP/PKA-dependant pathway (De Lamirande and Gagnon, 1998; Visconti and Kopf, 1998; Thundathil et al., 2003). Incubation of spermatozoa with NO donors increases

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intracellular cyclic guanosine monophosphate (cGMP) levels of the spermatozoa of bulls (Zamir et al., 1995) and humans (Revelli et al., 2001). NO stimulate hyperactivation motility (Herrero et al., 1995). Sperm hyper activation motility is promoted by exogenous addition of NO which may increase phosphorylation of flagella proteins (Thundathil et al., 2003; Harrison, 2004).

Hyper activation is crucial for sperm to penetrate the zona pellucid viscose environment that surrounds the oocyt (Gagnon and de Lamirande, 2006). First study on arginine was done in Iraq to stimulate *in vitro* the motility goat sperm (AL-Shaty, 2007). In spite of these, Artificial Insemination center in Iraq is responsible for processing of frozen semen in Iraq and the center depends on imported bulls to produce semen with high quality but for known or unknown reasons, some bulls produce ejaculates with low quality sometimes, especially in sperm motility, and for this reason these poor ejaculates were excluded from processing in this center which lead to economical loss. Therefore, this study was conducted as an attempt to: (1) Elevate the percentage of motility in these poor motile ejaculates by using different concentrations of arginine in the extender during different months; (2) explore the best concentration of arginine which gives best stimulation of sperm.

MATERIALS AND METHODS

This study was conducted between the periods of December, 2008 to July, 2009 and carried out at Artificial Insemination Center, Iraq, on 10 Holstein bulls imported from Australia. Semen was routinely collected from all bulls weekly with the aid of an artificial vagina. All bulls were of the same age (4 to 5 years) and were kept under identical conditions of management, feeding and watering was done throughout the study period. Poor motile ejaculates (estimated 30 to 55% of individual motility) were taken weekly and assessed as described by Chemineau et al. (1991).

A total of 114 ejaculates were studied during this period. After collection of semen, the sample was immediately brought to the laboratory and placed in a water bath at (37 to 38°C) and a phase-contrast light microscope used for evaluation of individual motility (Chemineau et al., 1991). Dead and abnormalities percentages were evaluated using Eosin-Nigrosin stain (Bearden et al., 2004) and acrosoma abnormalities percentage by using Haematoxylin-Eosin stain (WHO-NAFA, 2000). The poor ejaculates were diluted with Tris-yolk-fructose (1:10 diluted rate) and divided into six equal parts in six plastic test tubs, one used as control (contain diluents semen only without arginine) and the diluted semen in five test tubes were supplemented with five concentration of arginine 0.002, 0.003, 0.004, 0.005 and 0.006 M/ml.

Diluents semen in all test tube were sited at room temperature for 30 min, which is the optimum time for maximum arginine intake (Radany et al., 1981) and after 30 min, the diluents semen in the treated (five test tube which contain each one of test tube only one concentration of arginine) and the control test tubes were evaluated for individual motility, dead and abnormalities and acrosoma abnormalities by using the same technique for each of them.

Statistical analysis

Collected data were analyzed using (SAS, 2001) computer program (ANOVA) and Duncan Multiple Rang Test was performed to identify significant difference (at level $P < 0.05$) among the treatment means.

RESULTS

Tables 1, 2, 3 and 4 display changes in values of sperm motility, dead, abnormalities, abnormal in acrosoma of spermatozoa. From the tables, the following can be deduced.

Individual motility and livability of sperm

Table 1 revealed that due to a change in value of sperm motility, there were gradual increase in sperm motility which was parallel with increasing concentration of arginine with significant ($P < 0.05$) differences when compared with the control in all months of treatment, and the concentrations of arginine (0.005 and 0.006 M) produced highest values of survival motility time in all periods of preservation and in all months of treatment.

Dead percentage

Data presented in Table 2 shows changes in values of dead sperms. The data revealed that adding different concentrations of arginine decreases the percentage of dead sperms when compared with the control during the whole period of preservation in all months of this study, and best concentrations of arginine gave lower percentage of dead sperms in all periods of storage and was (0.005 and 0.006 M) with significant ($P < 0.05$) differences when compared with the control in all months of study.

Abnormalities percentage

Data of Table 3 exhibited changes in value of sperm abnormalities, the data showed that adding different concentrations of arginine decreases the percentage of abnormal sperms when compared with control during all months of this study, and best concentration of arginine gave lower significant ($P < 0.05$) difference percentage of abnormal sperms (0.005 and 0.006 M) when compared with the control in all months of treatment.

Acrosoma abnormalities

Table 4 revealed changes in value of acrosoma abnormalities, the data showed that adding different concentrations of arginine decreased the percentage of acrosoma abnormalities in sperms and best concentration of arginine gave lower significant ($P < 0.05$) difference percentage of acrosoma abnormalities in sperms (0.005 and 0.006 M) when compared with the control

Table 1. Effect different concentration of Arginine on Individual Motility (%) of poor motile bull semen.

Concentration of arginine (M)	December 2008 to July 2009					
	December	January	April	May	June	July
Control	45.57±1.99^{Da}	46.59±2.00^{Da}	43.40±1.44^{Da}	44.91±1.64^{Da}	40.50±1.35^{Db}	40.42±1.77^{Db}
0.002	49.64±1.77^{Da}	46.47±1.40^{Da}	46.40±1.56^{CDa}	46.66±1.79^{CDa}	41.00±1.75^{CDb}	42.35±1.99^{CDb}
0.003	57.85±1.35^{Ca}	55.00±1.05^{Ca}	51.40±1.69^{BCb}	50.83±1.91^{BCb}	41.50±1.81^{BCDc}	45.28±2.15^{BCc}
0.004	61.85±1.54^{BCa}	61.47±0.71^{BCa}	56.60±1.79^{ABb}	55.00±2.01^{ABbc}	52.00±1.87^{ABc}	53.21±2.43^{ABc}
0.005	71.07±1.07^{Aa}	69.12±0.77^{Aa}	61.20±2.27^{Ab}	59.79±2.13^{Abc}	55.00±4.47^{Ac}	57.14±2.65^{A}
0.006	69.12±0.84^{Aa}	68.94±0.86^{Aa}	61.20±1.85^{Ab}	59.58±1.99^{Abc}	60.00±1.64^{Ab}	55.71±2.50^{Ac}

Values are mean ± SE. Different capital letters represent differences (P < 0.05) between concentrations (columns). Different small letters represent differences (P < 0.05) between months (rows).

Table 2. Effect of different concentration of arginine on dead sperm (%) of poor motile bull semen.

Concentration of arginine (M)	December 2008 to July 2009					
	December	January	April	May	June	July
Control	12.25±0.55^{Ac}	12.32±0.53^{Ac}	17.70±1.45^{Ab}	19.23±1.15^{Aab}	20.00±0.95^{Aa}	20.11±1.16^{Aa}
0.002	10.35±0.55^{Ac}	10.38±0.54^{Ac}	14.90±1.44^{Ab}	17.39±1.14^{Aab}	17.20±0.94^{Aa}	16.32±1.16^{Aa}
0.003	10.35±0.54^{Ab}	10.44±0.52^{Ab}	13.86±1.44^{Ab}	16.37±1.14^{Aab}	16.17±0.96^{Aa}	16.28±1.16^{Aa}
0.004	10.39±0.55^{Ab}	9.47±0.53^{Ab}	14.80±1.45^{Aa}	16.41±1.16^{Aa}	16.22±0.94^{Aa}	15.28±1.12^{Aa}
0.005	8.35±0.54^{Bc}	7.41±0.53^{Bc}	10.80±1.44^{Bbc}	12.37±1.15^{Bab}	14.17±0.95^{Ba}	14.25±1.17^{Ba}
0.006	7.42±0.55^{Bc}	7.41±0.57^{Bc}	10.84±1.44^{Bbc}	12.07±1.15^{Bab}	14.10±0.98^{Ba}	13.08±1.17^{Ba}

Values are mean ± SE. Different capital letters represent differences (P < 0.05) between concentrations (columns). Different small letters represent differences (P < 0.05) between months (rows).

Table 3. Effect different concentration of arginine on abnormalities (%) of poor motile bull semen.

Concentration of arginine (M)	December 2008 to July 2009					
	December	January	April	May	June	July
Control	11.00±0.33^{Ac}	11.85±0.33^{Ac}	13.16±0.56^{Abc}	15.66±0.94^{Aab}	18.87±1.02^{Aa}	18.18±1.10^{Aa}
0.002	10.07±0.34^{Ab}	11.94±0.55^{Ab}	11.24±0.57^{Ab}	13.75±0.93^{Ab}	17.95±1.02^{Aa}	17.28±1.10^{Aa}
0.003	9.03±0.34^{Ab}	10.91±0.34^{Ab}	11.20±0.56^{Ab}	12.73±0.94^{Aab}	15.95±1.03^{Aa}	15.25±1.12^{Aa}
0.004	9.03±0.34^{Ab}	9.94±0.34^{ABb}	10.22±0.55^{Ab}	12.72±0.93^{Aab}	15.92±1.01^{Aa}	14.28±1.09^{Aa}
0.005	4.00±0.35^{Bb}	6.88±0.34^{Bb}	6.16±0.57^{Bb}	7.71±0.94^{Bab}	10.92±1.02^{Ba}	10.25±1.11^{Ba}
0.006	4.03±0.34^{Bb}	4.91±0.32^{Bb}	6.20±0.55^{Bb}	7.72±0.95^{Bab}	10.07±1.04^{Ba}	10.25±1.11^{Ba}

Values are mean ± SE. Different capital letters represent differences (P < 0.05) between concentrations (Columns). Different small letters represent differences (P < 0.05) between months (Rows).

Table 4. Effect different concentration of arginine on acrosoma abnormalities (%) of poor motile bull semen.

Concentration of arginine (M)	December 2008 to July 2009					
	December	January	April	May	June	July
Control	4.38±0.054^{Ac}	4.33±0.062^{Ac}	5.94±0.083^{Ab}	6.26±0.084^{Ab}	9.20±0.096^{Aa}	10.28±0.065^{Aa}
0.002	4.21±0.033^{Bb}	4.31±0.052^{Ab}	4.22±0.019^{Ab}	5.56±0.059^{Ab}	8.17±0.054^{ABa}	9.88±0.084^{Aa}
0.003	4.35±0.060^{Bc}	3.44±0.047^{Ac}	3.02±0.048^{Bc}	5.44±0.094^{Ab}	8.20±0.044^{ABa}	9.28±0.033^{ABa}
0.004	3.41±0.020^{Bb}	3.22±0.069^{Bb}	3.00±0.068^{Bb}	4.34±0.055^{Bb}	7.97±0.032^{Ba}	8.32±0.098^{BCa}
0.005	1.62±0.026^{Cc}	1.42±0.031^{Cc}	1.92±0.044^{Cc}	3.67±0.031^{Bb}	6.11±0.074^{Ca}	7.28±0.032^{CDa}
0.006	1.22±0.019^{Cc}	1.20±0.033^{Cc}	1.94±0.077^{C}	3.44±0.044^{Bb}	5.17±0.045^{Ca}	6.28±0.076^{Da}

Values are mean ± SE. Different capital letters represent differences (P < 0.05) between concentrations (columns). Different small letters represent differences (P<0.05) between months (rows).

in all months of treatment. Tables 1, 2, 3 and 4 revealed that L-arginine during different months increased the activation of motility and decrease of dead, abnormality and abnormal acrosoma of sperm during months of December and January when compared with other months, especially June and July. Furthermore, monthly variation in motility, dead, abnormality and abnormal acrosoma of sperm appears that months June and July gave lower significant ($P < 0.05$) difference in motility and high significant ($P < 0.05$) difference in percentage of dead, abnormality and abnormal acrosoma when compared with other months.

DISCUSSION

Results show that elevation in motility after using different concentrations of arginine may be due to the effect of arginine, which enhances sperm metabolism, thereby elevating glycolysis rate and synthesis of ATP, an energy rich compound essential for sperm motility (Keller and Polakoski, 1975; Patel et al., 1998). Moreover, Patel et al. (1998) mentioned that the addition of arginine in low concentrations increases the pH of cell suspension. Glycolysis was independent of pH over the range of 6.2 to 8.2 (Babcock et al., 1983; Patel et al., 1998), so Patel et al. (1998) concluded that addition of arginine at concentrations increases the metabolic activity of spermatozoa and lactic acid accumulation. Furthermore, Clancy et al. (1994) reported that the nitric oxide have a major role in stimulation of lactic dehydrogenize which was a key for metabolic process in sperm motility that may be presumably given as the sperm direct effects responses individual motility, as an end point.

Best concentrations of arginine gave lower percentage of dead sperms (0.005 and 0.006 M) with significant ($P < 0.05$) differences when compared with the control. 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 (Moncada and Higgs, 1993, Pacher et al., 2007); based on this, arginine has been known to increase generation of nitric oxide. Nitric oxide as a free radical has actually been shown to be a beneficial antioxidant against reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2) (Brown, 1999; Wink et al., 2001; Svegliati-Baroni et al., 2001), these free radicals are regularly released in mammalian cells during oxygen consumption and cause peroxidative damage to membrane phospholipids (Alvarez and Storey, 1989).

As discussed before, 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. This scavenging property gives nitric oxide a major intracellular and extracellular action against oxidative stress (Wink and Mitchell, 1998; Hogg and Kalyanaraman, 1999) that may provide protection against oxidation damage to bimolecular of sperm cell, which in turn elongates sperm life and decreases sperm abnormalities during storage. Results of this study showed that the percentage of sperm abnormalities in control of all months was between 3.85 and 9.18%.

Bearden et al. (2004) reported that every ejaculate of semen contain so morphologically, abnormal spermatozoa but the expected range is between 8 to 10%, which has no adverse effect on fertility. Also, results revealed that best concentrations of arginine gave lower percentage of acrosoma abnormalities (0.005 and 0.006 M). Increasing levels of superoxide radical led to increase in the membrane fluidity of the sperm membranes (Purohit et al., 1998). Membrane fluidity is reported to play an important role in sperm maturation in ram (Wolf and Voglmayr, 1984) and mice (Kumar, 1993), thus membrane fluidity is maintained by the controlled peroxidation of the membrane phospholipids by reactive oxygen species (Halliwell and Gutteridge, 1985; Jain et al., 1993). Uncapacitated spermatozoa produce low levels of NO, whereas under capacitation conditions, a time-dependent increase in NO synthesis has been observed (Belen Herrero et al., 2000), so the effect of arginine as an antioxidant may delay the aging process.

Benz et al. (2002) reported that since nitric oxide being a free radical along with the ability to scavenge other free radicals, it is placed in a Pivotal regulatory position. They speculated that in the absence of adequate nitric oxide release, other free radicals may initiate cells damage. The results of L-arginine during different months showed that increase in activation of motility in December and January might be due to a decrease in percentage of dead and abnormal sperms in these months when compared with other months. Several studies found evidence that variation in month of semen collection significantly affects seminal traits (Rathore, 1970).

This study showed that especially in hot months (June and July), there was low quality semen and this agrees with Curtis (1983) and Stalhammer et al. (1994). They reported that heat stress increased the percentage of sperm abnormalities on sperm cell (Ball and Peters, 2004; Carlsen et al., 2003; Noaks et al., 2001) and also that heat stress has effects on spermatogenesis; it means an effect on motility and concentration of sperma-tozoa (Bilboy and Jordan, 2009; Cholami et al., 2011).

Conclusion

Addition of 0.005 or 0.006 M/ml of arginine to semen diluents containing poor motile bull sperms improved sperm motility and decreased death, abnormality and defect in acrosoma of sperm especially during December and January. Also, results show that hot months (June and

July) caused low quality semen.

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