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Cryopreservation of Extended Bull Semen Using Aromated and Non-Aromated Amino Acids with Emphasis on Conception Rate

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Abstract: The aim of present study was to obtain the optimal concentration of non-aromated (L-cysteine, DL-alanine, glycine, L-glutamine) and aromated (L-tryptophan) amino acids for preserving bull semen characteristics and conception rate in a high level. Five mature cattle bulls were used as semen donors. Pooled semen were divided into aliquots to be added to a base extender (control, tris-citrate-fructose-egg yolk [TCFY]) and TCFY to which amino acid additives (DL-alanine, glycine and L-glutamine at concentrations of 25 and 100 mM/L), L-cysteine (2 and 10 mM/L) and L-tryptophan (0.5 and 5 mM/L) were added. Diluted semen was chilled and freezed in liquid nitrogen. Semen quality (motility, alive and abnormality%) and conception rate as a field test were practiced. Motility showed no difference in chilled diluted semen although significant difference ($P<0.0001$) was found in case of alive and abnormality%. While, all the quality parameters in case of freezed diluted semen were significantly ($P<0.01$) differed between amino acid groups as compared to the contol group. The conception rates after insemination was 81.48% for 10 mM L-cysteine, 81.25% for 100 mM DL-alanine, 90% for 25 mM glycine, 72.73% for 25 mM L-glutamine and 57.14% for 5 mM L-tryptophan as compared to control group 59.09%. It was concluded that addition of amino acids to bull semen extenders, especially non-aromated amino acids are more suitable for bull semen cryopreservation than aromated ones.

Key words: Cattle Bull · Non-Aromated Amino Acids · Semen Extender · Aromated Amino Acids

INTRODUCTION

The biological role of amino acids (e.g. glutamine, glycine, proline or histidine) in prevention of all damage during freezing comes from its effect as a non permeating cryoprotectant of many mammalian species including goat, ram and stallion [1-4].

The amino acid proline protect sperms against cold shock [5] and some other amino acids protect sperm against freezing stress [1, 6, 7].

Amino acids, especially glutamate are detected in higher concentration in rete testis of the seminiferous tubules of rat [8].

Addition of amino acids with glycerol improve post-thawing sperm motility in bull [9] as well as in goat [2, 10, 11]. Also, frozen-thawed ram sperm motility was found to be improved after the addition of glycine and proline at low concentration [3]. Addition of 80 mM glutamine significantly improved sperm motility as well as fertilizing ability in human and stallion [12].

The main objective of the current work was to study the efficiency of non-aromated (L-cysteine, DL-alanine, glycine, L-glutamine) and aromated (L-tryptophan) amino acids when added to bull semen extender for preserving semen characteristics and conception rate.

MATERIALS AND METHODS

Semen Collection and Initial Evaluation: Five mature cattle bulls maintained at Semen Freezing Center, General Organization for Veterinary Services Ministry of Agriculture, Abbasia, Egypt, were used as semen donors. Ejaculates were collected using an artificial vagina at weekly intervals for 5 weeks. Semen samples were initially evaluated for subjective sperm motility and sperm concentration. Ejaculates fulfilling minimum standard of sperm motility (70%) and sperm morphology (80%) were processed for freezing. The ejaculates were pooled in order to have sufficient semen for a replicate and to

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eliminate the bull effect. The semen was given a holding time for 10 minute at 37°C in the water bath before dilution.

Semen Processing: The reference cryopreservation extender (Control) was Tris-citric acid-fructose egg yolk (TCFY) diluent, prepared according to Foote [13].

The non-aromated amino acids (DL-alanine, glycine, L-glutamine) were obtained from Sigma Chemical Co.(USA) and added to the control extender at the concentrations of 25 and 100 mM/L [14], while L-cysteine was added at concentration of 2 and 10 mM as a modification for previous work [2, 15] concentrations. Aromated amino acid (L-tryptophan) was added at a concentration of 0.5 and 5 mM/L [16]. Eleven aliquots of semen were diluted at 37°C with each extender in order to provide concentration of 60 million sperm/ml. Extended semen was slowly cooled (approximately for 2 hrs) to 4°C and equilibrated for 4 hrs. Semen was packed into 0.5 ml polyvinyl French straws. After equilibrium periods, the straws were horizontally placed on a rack and frozen in a vapour 4 cm above liquid nitrogen for 10 minutes and were then dipped in liquid nitrogen.

Assessment of Semen Quality Parameters: The assessment was undertaken on neat semen, after cooling and freeze thawing of cattle bull spermatozoa. Frozen straws were thawed at 37°C/ 1 minute. The parameters studied were subjective semen characteristics (motility, alive and abnormality%) beside the conception rate in a field test practice.

Sperm Motility: Sperm motility% was subjectively assessed using microscope set at magnification of 400 and equipped with a heating plate (37°C). Visual motility was microscopically assessed with closed circuit television [17].

Sperm Abnormalities and Viability: This was established by Eiosin/Nigrosin staining [18]. All the semen evaluation was done by single person to avoid individual variations.

Conception Rate (CR): Two hundred and one cows were inseminated with extended bull semen to which different types and concentrations of amino acids were added. Forty four cows were inseminated with bull semen diluted with TCFY (control group). Pregnancy was confirmed by rectal palpation 2 months later after insemination. The inseminated cows were used via the cooperation with the Veterinary Services Organization in Fayoum Governorate. CR was calculated according to the equation:

$$CR = \frac{no.of\ conceived\ cows}{total\ no.of\ inse\min ated\ cows} x100$$

Statistical Analysis: data were analyzed using the SPSS (2005) computerized program v. 14.0 to calculate the analysis of variance (ANOVA) for the different parameters between additives replications. Number of straws examined represented the replicate (n=30). LSD was calculated for significant variance at $P<0.05$.

RESULTS

The effect of adding selected amino acids (L-cysteine, DL-alanine, glycine, L-glutamine and L-tryptophan) to semen extenders used for freezing of bull semen on sperm motility, alive, abnormalities after cooling and post-thawing were summarized in Tables 1 and 2. Also, the effect of adding these amino acids to semen extenders on conception rate percentage was illustrated in Table 3.

Table 1: Effect of different concentrations of selected amino acids on some characteristics of bull spermatozoa after cooling (Mean±SE)

	L-cysteine		DL-alanine		Glycine		L-glutamine		L-tryptophan			
Parameter	2 mM/L	10 mM/L	25 mM/L	100 mM/L	25 mM/L	100 mM/L	25 mM/L	100 mM/L	0.5 mM/L	5 mM/L	Control	Sig
Motile%	78.33±4.41	81.67±1.67	85.00±2.89	81.67±3.33	81.67±1.67	88.33±1.67	80.00±5.77	81.67±4.41	83.33±3.33	83.33±3.33	81.67±1.67	0.793
Alive%	71.67^{c}±1.67	81.67^{d}±1.67	85.00^{bcd}±2.89	83.33^{cd}±1.67	83.33^{cd}±1.67	88.33^{abc}±1.67	83.33^{cd}±1.67	91.67^{a}±1.67	91.67^{a}±1.67	90.00^{ab}±0.00	81.67^{c}±1.67	0.000
Abnormality%	14.67^{c}±0.33	18.67^{a}±0.67	15.67^{c}±0.33	14.33^{c}±0.33	15.33^{c}±0.33	15.67^{c}±0.33	14.67^{c}±0.33	17.00^{b}±0.58	12.00^{d}±0.58	10.33^{e}±0.33	14.33^{c}±0.33	0.000

Within row, means with different alphabetical superscripts are significantly different at least at P<0.05.

Table 2: Effect of different concentrations of selected amino acids on some characteristics of bull spermatozoa after freezing (Mean±SE)

	L-cysteine		DL-alanine		Glycine		L-glutamine		L-tryptophan			
Parameter	2 mM/L	10 mM/L	25 mM/L	100 mM/L	25 mM/L	100 mM/L	25 mM/L	100 mM/L	0.5 mM/L	5 mM/L	Control	Sig
Motile%	38.75^{d}±0.65	48.33^{bc}±0.71	40.00^{d}±3.26	41.67^{d}±1.88	43.33^{cd}±1.42	30.00^{e}±2.46	44.00^{bcd}±1.72	57.50^{a}±0.75	49.00^{b}±2.20	56.25^{a}±1.25	47.08^{bc}±0.57	0.000
Alive%	51.67^{b}±1.67	51.67^{b}±1.67	58.33^{ab}±1.67	58.33^{ab}±1.67	58.33^{ab}±1.67	51.67^{b}±1.67	60.00^{a}±2.89	60.00^{a}±2.89	51.67^{b}±1.67	51.67^{b}±1.67	53.33^{ab}±3.33	0.013
Abnormality%	15.67^{b}±0.33	12.33^{cd}±0.33	11.33^{de}±0.67	10.67^{e}±0.67	15.33^{b}±0.33	15.67^{b}±0.33	15.67^{b}±0.33	15.67^{b}±0.33	13.67^{c}±0.33	13.67^{c}±0.33	19.67^{a}±0.88	0.000

Within row, means with different alphabetical superscripts are significantly different at least at P<0.05

Table 3: Conception rate of cows inseminated with extended bull semen with different concentrations of selected amino acids

Extender addition	Conception rate
Control	59.09
Different amino acids	
Non-aromated amino acids	
L-cysteine (2mM/L)	61.54
L-cysteine (10 mM/L)	81.48
DL-alanine (25 mM/L)	60.00
DL-alanine (100 mM/L)	81.25
Glycine (25 mM/L)	90.00
Glycine (100 mM/L)	46.15
L-glutamine (25 mM/L)	72.73
L-glutamine (100 mM/L)	70.00
Aromated amino acids	
L-tryptophan (0.5 mM/L)	33.33
L-tryptophan (5 mM/L)	57.14

The addition of 2 mM and 10 mM L-cysteine to the control freezing extender lead to a significant (P<0.0001) decrease in sperm abnormalities after freezing. The addition of DL-alanine 25 mM significant (P<0.0001) improved the percent of alive sperm after cooling and significant (P<0.0001) decreased sperm abnormalities after freezing. Glycine added in a concentration 25 and 100 mM caused significant (P<0.0001) increase in percent of alive sperms and after cooling significant (P<0.0001) decrease in sperm abnormalities after freezing.

L-glutamine added in concentration 25 and 100 mM exhibited significant (P<0.0001) improvement in percent of alive sperms after cooling and 100 mM concentration revealed significant (P<0.0001) improvement in sperm motility, alive and abnormalities after freezing.

L-tryptophan addition 0.5 and 5 mM significantly (P<0.0001) improved alive and abnormalities after cooling and motility and abnormalities after freezing.

DISCUSSION

Cryopreservation of cattle semen often induce an additional source for reactive oxygen species (ROS) attack on sperm due to decreased activities of antioxidant enzymes and the sperm membrane become more susceptible to lipid peroxidation [19] which affect the membrane permeability [20].

Addition of amino acids (L-cysteine, DL-alanine, glycine and L-glutamine) to semen extenders significantly improved semen characteristics and conception rate percentage in cattle inseminated with this extended semen. This improvement is due to the best protection against cold shock [21], higher superoxide dismutase and catalase activity [9], increased catalase activity and vitamin E levels [22] and reduced DNA damage [11]. However, amino acid concentration if increased above certain level, a deleterious effect on cryopreservation is originated due to osmotic shock [23].

The L-tryptophan (aromated amino acid) supplemented group gave no improvement in conception rate, this is due to the higher concentration of the used L-tryptophan where L-amino acid oxidase produced from dead sperms reacts with L-tryptophan producing the toxic hydrogen peroxide and ammonium ions [16]. This toxic effects occurs during the journey of sperm in the female genital tract giving low conception rate.

It was concluded that amino acid additives to bull semen extenders, especially non-aromated amino acids are more suitable for bull semen cryopreservation than aromated ones.

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