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The effects of extenders containing proline and glutamine on oxidative stress and motion parameters of stallion semen during cold storage

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ABSTRACT

This study examined the effects of skim-milk based extenders supplemented with proline and glutamine on motility, lipid peroxidation and enzymatic antioxidant status of cooled-stored equine sperm and determined the role of seminal plasma as well. The semen was collected with artificial vagina. In experiment 1, native semen was diluted in skim-milk based extender containing 5mM glutamine and 3mM proline, stored at 5°C and analyzed at 4, 24 and 48 hours storage for motion parameters. In experiment 2, semen was centrifugated, sperm pellet resuspended in the extenders and stored at 5°C for 4 hours to determine motion parameters. The level of catalase, glutathione peroxidase activity and malondialdehyde formation was determined for all samples at 4 hours. Glutamine and proline significantly preserved the percentage of motile sperm (76.5 ± 2.7 and 79.4 ± 1 vs 69 ± 1.4), increased the progressive motility of cold-stored semen (66.1 ± 2.5 and 73.7 ± 2.9 vs 56.2 ± 1.4), increased catalase activity, and decreased malondialdehyde. However these effects were disappeared after seminal plasma removal. We conclude that glutamine and proline would amplify the antioxidant activity of equine cold-stored semen and preserve its motility. This effect seems to be related to interactions with seminal plasma.

Keywords

glutamine, proline, cold storage, stallion, semen, antioxidant activity

Abbreviations

SP: seminal plasma
MDA: malondyaldehyde
CAT: catalase
GPX: glutathione peroxidase

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Introduction

The use of artificial insemination in world quine industry eliminates the detrimental effects of animal transportation and transmission of diseases, as well as providing more opportunities for breeders to make use of genetically superior stallions [1]. The techniques of cooling and transportation of semen, as well as cryopreservation allows artificial insemination to take place, irrespective of the location and availability of stallions. However, it is obvious that the fertility rate for artificial insemination is lower than that for natural service [2].

Oxidative stress is an important factor associated with the decline in fertility of stored semen. During refrigerating storage, the number of viable sperms decreases [3], fertility rate declines [4], and damaging intracellular reactive oxygen species (ROS) is found to be accumulated [5, 6]. Semen is composed of spermatozoa and seminal plasma, endowed with enzymatic and non-enzymatic defense mechanisms against oxidative stress [7]. Seminal plasma is a complex mixture of organic and inorganic substances with controversial effects on sperm properties [8, 9], and is thought to influence the fertility outcome of the cooled-shipped semen. Some studies have revealed that seminal plasma exerts deleterious effects on sperm during cooling transportation, and that the separation of seminal plasma reduces those effects and maximizes post-cooling motility [10, 11]. Removal of seminal plasma has been described to result in increased percentage of motile sperm and membrane and chromatin intactness, both during refrigerated storage, as well as delayed cryopreservation [12-15]. Nevertheless, some studies propose that removal of seminal plasma eliminates the potential protective capacity provided by the SP [16, 17]. This potential can be related to antioxidant properties and ROS scavenging capability of seminal plasma, which can even be enhanced through interacting with semen extenders [18]. However, centrifugation of cooled sperm is thought to be detrimental to sperm motility [13, 19]. The equipment for seminal plasma removal is not always available at the location of semen collection. In cases where farm is far from laboratory, semen must be cooled-shipped and it is necessary to preserve equine semen in extender for several hours without removing seminal plasma. Hence, during transportation, sperms are exposed to seminal plasma. In these cases the modification of semen extender composition has been proposed as an alternative to separation of SP [20]. The protection of cooled equine semen against oxidative stress and loss of motility might be improved by adding some specific components to extenders.

Some amino acids are known to be involved in protecting several types of animal cells against hypothermia [21-23]. The amino acid proline can preserve membrane structure of sperm cells [24], reduces LPO, protects against free radical damages, inhibits intracellular ice formation and influences the motility and velocity [25, 26]. Likewise, amino acid glutamine is known to play a regulatory role in several cell-specific processes [27, 28]. Based on these information, glutamine and proline have been proposed as efficient additives in semen extenders to function as cryoprotectant in several species [24, 27-31]. However, the results of studies considering these amino acids as cryoprotectant have been controversial in terms of improving either the maintenance of motility or the fertility of cooled semen.

The hypothesis of this study was that addition of proline and glutamine to extender could improve the longevity of stallion sperm motility during cold storage. The study was designed to investigate whether the skim-milk based extender supplemented with proline and glutamine could preserve stallion semen for 48 hours of cold transportation. Moreover, the effects of seminal plasma on the enzymatic antioxidant status of extended semen in the presence of these amino acids were evaluated. It will be determined whether the inclusion of glutamine and proline in the extender in the presence and absence of seminal plasma, have any effect on the motility, lipid peroxidation, and enzymatic antioxidant status of stallion sperm during storage at 5°C. Such studies can help breeders to optimize protocols for the transportation of stallion's spermatozoa, which must be maintained for long intervals.

Results

Experiment 1

Mean percent motile and progressive sperm motility of raw semen at the time of collection were 79.5 ± 1.2 and 73.2 ± 1.5, respectively. Effects of semen treatments on the percentage of motile sperm and progressive motility of stallion semen stored up to 48 hours are presented in Table 1. In each group of extenders, (E1, E2, and E3) all the parameters at 4 hours storage were significantly different relative to 24 and 48 hours storage ($p < 0.05$). At 4 hours storage, the percent motile sperm of E2 (76.5 ± 2.7) and E3 (79.4 ± 1.9) were significantly higher than E1 (69 ± 1.4) ($p < 0.05$). The progressive motility of E2 (66.1 ± 2.5) and E3 (73.7 ± 2.9) were significantly higher than E1 (56.2 ± 1.4) ($p < 0.05$). At 24 and 48 hours storage, percent motile sperm and progressive motility of E2 an E3 were significantly higher than E1 ($p < 0.05$) (Table 1). At 4 hours storage, the MAD, BCF and STR of E2 and E3 were significantly decreased compared to E1. The VCL and LIN of E3 were significantly decreased compared

Table 1
The Effects of aminoacids on the percentage of motile sperms and progressive motility of stallion semen cooled to 5°C up to 48 hours.

		Extenders		
		E1	E2	E3
Motion parameters	Time (h)			
Percent motile sperm (%)	4	69 ± 1.4^{a1}	76.5 ± 2.7 ^{a2}	79.4 ± 1.9^{a2}
	24	20 ± 0.9^{b1}	33.9 ± 1.2^{b2}	40.7 ± 0^{b2}
	48	11.2 ± 0.7^{b1}	30.2 ± 0.8^{b2}	34 ± 0.3^{b2}
Progressive motility (%)	4	56.2 ± 1.4^{a1}	66.1 ± 2.5^{2a}	73.7 ± 2.9^{a2}
	24	10.8 ± 1^{b1}	15.8 ± 1.2^{b}	21.5 ± 0 ^{b2}
	48	10.1 ± 0.4^{b1}	14.1 ± 1.8^{b2}	13.9 ± 2.2 ^{b2}

Different numbers in each row and different letters in each column are indicative of statistically significant differences at $p < 0.05$. E1: control extender; E2: the control extender supplemented with 5 mM glutamine, E3: the control extender supplemented with 3 mM proline. Values are mean percentages ± SEM of ten split ejaculates (2 ejaculates x 5 stallions). The control extender contained: INRA82 +2% egg yolk + 2 5% (v/v) glycerol.

to E1 and E2. At 24 and 48 hours storage, differences in kinematic parameters between three groups were not statistically significant (Table 2).

Experiment 2

As shown in Table 3, after 4 hours storage, the percent motile sperm and progressive motility of equine spermatozoa in E4 were not different with E5 and E6. At 48 hours of storage the motility parameters of E4, E5 and E6 were reduced to near zero.

The results shown in Table 4 indicate that the content of MDA is significantly different in E4 (0.7 ± 0.08 nmol/mg protein) relative to E5 (0.18 ± 0.08 nmol/mg protein) and E6 (0.39 ± 0.04 nmol/mg protein) ($P < 0.05$). The MDA of E4 is also significantly higher than E2 (0.35 ± 0.09 nmol/mg protein) and E3 (0.3 ± 0.03 nmol/mg protein). There is no difference between MDA content of E1 with E2, E3 and E4. The activity of GPX remained unchanged in all groups. The activity of CAT enzyme was significantly different between E1 (35.4 ± 7.8 KU/ mg protein), E2 (166.2 ± 21 KU/ mg protein) and E3 (154.6 ± 60 KU/ mg protein). Differences between E4, E5, and E6 were not statistically significant (Table 4).

Discussion

In the present study, the effects of glutamine and proline on motion and kinematic parameters, as well as lipid peroxidation and antioxidant capacity of cooled stallion semen, and their relationship to seminal plasma were tested. The results of experiments 1 and 2 revealed that addition of proline and glutamine significantly preserved the motion parameters of cooled stallion semen over time. In the absence of SP, none of the motion parameters were affected by two used amino acids.

The positive effects of glutamine and proline on stallion sperm subjected to cryopreservation have been reported [27, 30, 32]. The present study revealed that these two amino acids improved the motion parameters of cold-stored native semen, but in groups lacking seminal plasma, motility was unaffected. It was previously proven that in the absence of seminal plasma, addition of extenders composed of nonfat, dried skim milk solids and glucose provide adequate support for the preservation of spermatozoa motility [10]. Our study showed compensatory effect for proline and glutamine in preservation of equine spermatozoa motility in the presence of seminal plasma.

Although the reduction in spermatozoa1 motion parameters in extended stallion semen after 24 hours of cold storage has previously been reported [11], the role of seminal plasma in motion characteristics of sperm in different studies is contradictory. The reduction [9], improvement [10, 11, 33] and no change [34] in spermatozoa1 motility after seminal plasma removal have been reported. Two studies [35, 36] demonstrated no effect for SP during cooling of the stallion semen up to 12 and 24 hours, respectively. Whereas another study [12] speculated that long exposure to seminal plasma in cooled stallion sperm may be responsible for the irreversible loss of motility and DNA degradation. It is thought that centrifugation for SP removal eliminates decapacitating factors in SP [37], resulting in premature capacitation [38]. This has been related to the protective roles of some components of seminal plasma, which is variable in different stallions [39]. The results of Moor et al., indicated that prolongation of the semen incubation time causes undesir-

Table 2
Kinematic parameters of stallion semen extended in the control extender (E1), and extenders supplemented with 5 mM glutamine (E2) and 3 mM proline (E3), stored at 5°C for 48 hours.

		Extenders		
		E1	E2	E3
Kinematic parameters	Time (h)			
VCL (µm/s)	T4	77.8 ± 2.3 ^{a1}	73.2 ± 13.5 ^{a1}	69.3 ± 3.2 ^{a2}
	T24	20.7 ± .5 ^{b}	23 ± 2.3 ^{b}	22.8 ± 0 ^{b}
	T48	18.9 ± .5 ^{b}	20.3 ± .5 ^{b}	19.2 ± .8 ^{b}
VSL (µm/s)	T4	41.6 ± 2 ^{a1}	40 ± 7.8 ^{a1}	39.3 ± 1.3 ^{a1}
	T24	6 ± .1 ^{b}	5.9 ± .4 ^{b}	6 ± 0 ^{b}
	T48	4.4 ± .3 ^{b}	5 ± .7 ^{b}	5.2 ± .6 ^{b}
VAP (µm/s)	T4	47.5 ± 2 ^{a1}	46.8 ± 8.9 ^{a1}	46.4 ± 1.4 ^{a1}
	T24	10.2 ± .3 ^{b}	9.9 ± .4 ^{b}	10.8 ± 0 ^{b}
	T48	8.3 ± .4 ^{b}	9.4 ± .6 ^{b}	8.6 ± .5 ^{b}
MAD (°)	T4	46.1 ± 4.1 ^{a1}	24.4 ± 6 ^{a2}	22.8 ± 1.8 ^{a2}
	T24	2.5 ± .2 ^{b}	2.6 ± .1 ^{b}	2.5 ± 0 ^{b}
	T48	2.3 ± .1 ^{b}	2.5 ± .1 ^{b}	3.2 ± .5 ^{b}
ALH (µm)	T4	3.1 ± .1 ^{a1}	3.2 ± .3 ^{a1}	3.1 ± .1 ^{a1}
	T24	1.6 ± .1 ^{b}	1.7 ± .1 ^{b}	1.7 ± 0 ^{b}
	T48	1.5 ± .04 ^{b}	1.6 ± .06 ^{b}	1.6 ± 0.1 ^{b}
BCF (Hz)	T4	1.5 ± .1 ^{a1}	7 ± .2 ^{a2}	.8 ± .05 ^{a2}
	T24	.03 ± 0 ^{b}	.03 ± 0 ^{b}	.02 ± 0 ^{b}
	T48	.02 ± 0 ^{b}	.02 ± 0 ^{b}	.03 ± .02 ^{b}
LIN (%)	T4	48.4 ± 1.6 ^{a1}	49.2 ± 2.4 ^{a1}	43.9 ± 1.1 ^{a2}
	T24	24.2 ± .7 ^{b}	19.5 ± 0 ^{b}	24.3 ± 0 ^{b}
	T48	18.6 ± .8 ^{b}	23.7 ± 3.$^{5\,b}$	23.8 ± 2.9 ^{b}
WOB (%)	T4	59.2 ± 1.4 ^{a1}	62.4 ± 2.1 ^{a1}	57 ± 2.1 ^{a1}
	T24	40.5 ± 2.7 ^{b}	34.1 ± .9 ^{b}	41.8 ± 0 ^{b}
	T48	33.3 ± 1.1 ^{b}	40.6 ± 2.9 ^{b}	37.2 ± 2.3 ^{b}
STR	T4	74.2 ± 4.4 ^{a1}	69.9 ± 1.3 ^{a2}	63.3 ± .7 ^{a2}
	T24	39.2 ± .9 ^{b}	37.7 ± 2.6 ^{b}	42.3 ± 0 ^{b}
	T48	32.9 ± 1.3 ^{b}	39.4 ± 4.2 ^{b}	38.3 ± 3.$^{3\,b}$

Different numbers in each row and different letters in each column are indicative of statistically significant differences at $p < 0.05$. Values are mean percentages ± SEM of ten split ejaculates.

Table 3
The effects of SP and extenders supplemented with amino acids on motion parameters of 4 hours cold storage of equine sperm at 5°C.

	Extenders					
	E1	E2	E3	E4	E5	E6
Motion parameters						
Percent motile sperm (%)	69 ± 1.4[a]	76.5 ± 2.7[b]	79.4 ± 1.9[b]	71.54 ± 1.8[a]	71.6 ± 3.2[a]	72.23 ± 3.4[a]
Progressive motility (%)	56.2 ± 1.4[a]	66.1 ± 2.5[b]	73.7 ± 2.9[b]	73± 2 ± 0.0[b]	72.2 ± 4.3[b]	73.6 ± 3.2[b]

Different subscriptions: Significant difference between control and supplemented groups at $p < 0.05$ in each row. Values are mean percentages ± SEM of ten split ejaculates (2 ejaculates x 5 stallions).

Table 4
Effects of control extenders supplemented with aminoacids on lipid peroxidation and antioxidant activity of cooled stallion semen and spermatozoa.

	Extenders					
	E1	E2	E3	E4	E5	E6
parameters						
MDA (nmol/mg protein)	.49 ± 0.1[a,b]	0.35 ± 0.09[b]	0.3 ± 0.03[b]	0.7 ± 0.08[a]	0.18 ± 0.08[b]	0.39 ± 0.04[b]
GPX (IU/mgr protein)	2713 ± 28	3116 ± 225	2731 ± 124	3286 ± 378	2471 ± 40	3053 ± 357
Catalase (KU/ mgr protein)	35.4 ± 7.8[a]	166.2 ± 21[b]	154.6 ± 60b	74.5 ± 65	65 ± 9.6	73 ± 27

Different subscriptions: Significant difference between control and supplemented groups at $p < 0.05$ in each row. Values are mean ± SEM of ten split ejaculates (2 ejaculates ×5 stallions). the levels of malondialdehyde formation (MDA) as an indicator of lipid peroxidation and the activity of glutathione peroxidase (GPx), catalase (CAT) and Superoxide Dismutase (SOD) as indicators of antioxidant activity are presented.

able effects, probably due to the presence of seminal plasma [40]. Our results revealed that the decrease in motion parameters of stallion sperm during cold storage was compensated by addition of extenders containing aminoacids. and this positive effect of aminoacids became evident only in the presence of seminal plasma. It seems that the interaction between proline and glutamine with seminal plasma is responsible for such compensatory effects.

Based on our results, glutamine and proline enhanced CAT activity in the presence of SP. This is when the motility of sperm is improved. Interestingly, MDA was decreased in groups containing aminoacids irrelevant to seminal plasma. Seminal plasma contains organic and inorganic substances such as high levels of enzymatic and non-enzymatic antioxidants. It is indicative of an important role that SP plays in the preservation of spermatozoa against degradation caused by ROS [41, 42]. However, It has been demonstrated that the interactions of an enzyme or a protein of bulbourethral origin in SP [43] with egg yolk [44] or milk [20] of extenders is detrimental to sperm. On the other hand, stallion seminal plasma is characterized by high sodium concentration, which is thought to induce spontaneous lipid peroxidation of the sperm membrane, resulting in decreased membrane fluidity and fertilizing capacity [45, 46]. Thus, it is expected that high concentration of seminal plasma would be deleterious to sperm cells subjected to cooling and storage [33, 34]. In order to solve this problem and to maximize post-cooling motility, separation of seminal plasma and addition of semen extender have been suggested [10, 11]. The single semen extender induces a compensatory effect on non-enzymatic antioxidant activity after seminal plasma removal. This is due to the composition of the extender, which shows enough antioxidant activities for semen protection against oxidative stress [47]. On the other hand removal of seminal plasma eliminates the potential protective capacity provided by SP [16], and centrifugation may not be easily available in the field as well, so the modification of extender composition to prevent enzymatic reactions might be more acceptable for cooling storage instead of SP removal.

Several studies have shown that supplementation of semen extenders with glutamine and proline can improve total sperm motility of cooled ram sperm, protect sperm cells against free-radical-induced damage, and provide cryoprotection to ram sperm by minimizing lipid peroxidation both at pre-freeze and post-thaw semen [48]. Rudolph [49] stated that proline may interact with phospholipid bilayers and stabilize the membrane structures during freezing. Similarly, in this study, inclusion of glutamine and

proline to the extender reduced malondyaldehyde formation, which was concomitant with positive changes in motion parameters, which shows that lipid peroxidation was inhibited by proline and glutamine during cooling storage. This was confirmed by another study showing that the positive role of proline for the motility of freeze-thawed stallion spermatozoa [30]. In the same way, Pagl [50] explained the dysfunctional role of plasma membrane and mitochondrial membrane in the loss of cooled-stored sperm motility.

We also found that CAT activity, as well as motion parameters increase in cooled semen in the presence of proline and glutamine with SP. Bucak [23] hypothesized an antioxidative effect for glutamine through increasing CAT activity, and Baumber [51] demonstrated that CAT enzyme prevents negative effects of ROS on motility and DNA fragmentation of equine sperm. On the other hand, Kancofer [18] proposed that the interactions among components of extenders and antioxidative enzymes of seminal plasma boost the antioxidative/oxidative capacity of stallion seminal plasma. This means that some factors in extenders influence activities of antioxidant enzymes present in seminal plasma. In addition, amino acids in combination with glycerol affect calcium ATPase of sperm cells [52], as well as the phosphate groups of sperm plasma membrane phospholipids for protection against thermal shock [53]. In this research, we found that removal of seminal plasma significantly conversed the effect of glutamine and proline on antioxidant enzymes. Therefore, it appears that these aminoacids need a certain amount of seminal plasma for enhancing enzymatic antioxidative capacity during cooling storage, which preserve motion parameters of cold-stored sperm as well.

It is concluded that the skim-milk extender containing glutamine and proline amplify the antioxidant activity of equine cold-stored semen, as well as motility preservation. This effect seems to be related to interactions with seminal plasma.

Material and methods

Semen collection

Five fertile stallions aged between 4-9 years were used in this study. They were on a routine semen collection schedule and fed balanced diet. Semen samples were collected using artificial vagina, and two ejaculates were obtained from each stallion. They had already been included in previous semen cryopreservation program, and had been shown to have good semen freezability [30].

Reagents

Unless otherwise indicated, all reagents used in the experiments were obtained from Sigma-Aldrich Company (St. Louis, MO).

Table 5
Osmotic pressure and pH values of the extender media used for cooling storage of semen samples.

parameters	Osmotic pressure (mOsm/kg)	pH
BM	6.8	336
BM+ 5 mM glutamine	6.8	338
BM+ 3 mM proline	6.8	337

BM: INRA82 medium+ 20mM HEPES/L + 2% centrifuged egg yolk and 2.5%, v/v glycerol

Preparation of extenders and semen

All extender media were derived from basal medium (BM), composed of INRA 82 medium (0.5 L saline solution: 25 g glucose, 1.5 g lactose, 1.5 g raffinose, 0.25 g sodium citrate dihydrate, 0.41 g potassium citrate, 50,000 IU penicillin, 50 mg gentamycin and 0.5 L skim milk) reaching final concentration of 2% (v/v) centrifuged egg yolk, 20 mM HEPES and 2.5% v/v glycerol (pH: 6.8) [32].

Aliquots of BM extender were supplemented with different concentrations of proline and glutamine (Merck corporation, Germany): 0 (E1 and E4 as control group), 5 mM glutamine (E2 and E5) and 3 mM proline (E3 and E6) (pH: 6.6- 6.8). Osmotic pressure of the extender media was measured with an automatic osmometer (OSMOMETER 800c l, SLAMED, Germany) (Table 5).

A total of ten Ejaculates were collected using a Missouri-model artificial vagina. After removing the gel fraction from the ejaculate by filtration through gauze, the gel-free portion of the ejaculate was evaluated for volume and progressive motility, as well as measuring spermatozoa concentration by a hemocytometer. One part of the raw semen was diluted 1:1 by adding: basal medium as the control (E1), basal medium containing 5mM glutamine (E2) and basal medium containing 3mM proline (E3). Then, it was cooled to 5°C, and stored for 48 hours.

The other part of native semen was submitted to centrifugation for seminal plasma removal, and spermatozoa were resuspended to a concentration of 25×10^6 motile sperms/ml in three types of extenders: basal medium as the control (E4), basal medium containing 5mM glutamine (E5), and basal medium containing 3mM proline (E6). Then, it was cooled to 5°C and stored for about 4 hours.

Experimental design

In Experiment 1, routine semen analysis was performed in cooled semen E1, E2 and E3 at 4, 24 and 48 hours storage at 5°C.

In experiment 2, changes in malondyaldehyde (MDA) formation (TBARS concentration as an indicator of lipid peroxidation), enzymatic antioxidant activities of catalase and glutathione peroxidase as well as CASA parameters for all 6 groups were monitored in cooled semen samples after 4 hours storage.

Evaluation of Semen Motion and kinematic parameters

Semen quality analysis was performed using the CASA system (HFT CASA- Houshmand Fanavar- Tehran- Iran) to evaluate the sperm motion parameters. Sperm samples (10 mL) were diluted in 0.5 mL of phosphate-buffered saline (PBS) and subjected to CASA. Five field images (minimum of 500 cells) were randomly selected and analyzed for the following parameters: motile sperm and progressively motile sperm percentages, as well as sperm kinematic parameters, including amplitude of lateral head displace-

ment (ALH), beat cross frequency (BCF), curveilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN = VSL/VCL), mean angular displacement (MAD), wobble (WOB= VAP/VCL) and straightness (STR = VSL/VAP).

Biochemical analysis

Biochemical assays were performed on the sperm samples homogenized by an ultrasonic homogenizer (FAPAN, Iran); then, total protein level of sperm was measured using a Bio-Rad protein assay kit (Bio Rad, Hercules, CA, USA). The levels of MDA, GPX and CAT were measured in protein content of sperm cells.

Concentration of malondialdehyde, as indices of the LPO, was determined colorimetrically using the method of Buege & Aust [54]. Briefly, 0.1 mL of sperm homogenate was treated with 2 mL of TBA–TCA–HCl reagent, placed in water bath for 15 min, cooled, centrifugated and then the absorbance of supernatant was measured against reference blank at 535 nm (Unico 2100, United Products Instruments, Inc., Dayton, NJ, USA). Concentrations were calculated using an extinction coefficient of 1.56×10^5 mol/L/cm. The MDA concentration was expressed in nmol/ml.

Activity of glutathione peroxidase was estimated according to the method of Lawrence & Burk [55], in which the reaction mixture containing potassium phosphate buffer, EDTA, sodium azide, NADPH, glutathione reductase and reduced glutathione was incubated for 5 min at 25°C, and after adding H_2O_2 and sperm homogenate, the absorbance changes at 340 nm was monitored for 1 min. One unit of GPX activity was reported as µmol NADPH consumed per min per mg sperm protein, using the appropriate molar absorptive coefficient for NADPH (6220 mol/L/cm).

Activity of sperm catalase was assayed according to the method of Goth [56]. Briefly, sperm homogenate samples were incubated in the reaction mixture composed of potassium phosphate buffer and H_2O_2, and 60 seconds later ammonium molybdate solution was added to terminate the reaction. The absorbance of the yellow color of this complex was measured at 405 nm. One unit of catalase activity was defined as the amount of enzyme that catalyzes the decomposition of 1 µmol of hydrogen peroxide per minute.

Statistical Analysis

Data were expressed as the mean ± SEM. Statistical analysis was carried out using SPSS 14.0 (SPSS, Chicago, IL, USA). The normality and homogeneity of variables were confirmed using Shapiro–Wilk and Levene's tests, respectively. One-way analysis of variance followed by Tukey's post hoc test was performed to compare the differences between groups. The repeated measure analysis of variance was performed for compression of indices during the cold storage period. The differences were considered significant at $p < 0.05$.

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Author Contributions

Conceived and designed the experiments: ND. Performed the experiments: ND, AK. Analyzed the data: AK, AA. Research space and equipment: ND, AK, AA. Wrote the paper: ND.

Conflict of Interest

We wish to confirm that there is no known conflict of interest associated with this publication.

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Abstracts (in Persian)

تاثیر محیط نگهدارنده حاوی گلوتامین و پرولین بر روی استرس اکسیداتیو و پارامترهای حرکتی منی نریان در زمان نگهداری به صورت سرد شده

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چکیده

این مطالعه اثرات محیط نگهدارنده غنی شده با اسیدآمینه های پرولین و گلوتامین را بر تحرک اسپرم، پراکسیداسیون لیپیدی و وضعیت آنتی اکسیدانی منی سرد شده اسب مورد بررسی قرار داد و همچنین نقش پلاسمای منی را نیز مشخص کرد. منی با واژن مصنوعی جمع آوری شده و با محیط نگهدارنده حاوی ۵ میلی مول گلوتامین و ۳ میلی مول پرولین مخلوط شده و در دمای ۵ درجه سانتیگراد نگهداری شدند و در آزمایش اول، پارامترهای حرکتی در زمان های ۴، ۲۴ و ۴۸ ساعت مورد آنالیز قرار گرفت. در آزمایش دوم، پلت حاصل از سانتریفیوژ منی ، در محیط های پایه و محتوی آمینو اسیدها مخلوط شده و پس از چهار ساعت نگهداری در دمای ۵ درجه سانتی گراد، مورد ارزیابی پارامترهای حرکتی قرار گرفت. سطح فعالیت کاتالاز، گلوتاتیون پراکسیداز و تشکیل مالون دی آلدهید برای تمامی نمونه ها در زمان ۴ ساعت سردسازی اندازه گیری شد. نتایج نشان داد که گلوتامین و پرولین بطور مشخصی باعث حفظ پارامترهای حرکتی اسپرم نریان شدند، فعالیت کاتالاز را افزایش داده و تولید مالون دی آلدهید را کاهش دادند. با این وجود پس از حذف سمینال پلاسما، این اثرات آمینواسیدها نیز مشاهده نشد. نتیجه گیری می شود که گلوتامین و پرولین فعالیت آنتی اکسیدانی منی سردشده اسب را تقویت کرده و تحرک اسپرم را حفظ می کنند و به نظر می رسد این نقش به اثرات متقابل با پلاسمای منی ارتباط داشته باشد.

واژگان کلیدی

پر، فعالیت آنتی اکسیدان، سیمن نریان، سردسازی، تامین