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Research Article

EFFECT OF ADDITION OF ANTIOXIDANTS IN THE EXTENDER TO FREEZE BOAR SEMEN IN TWO TYPES OF STRAWS ON SPERMA QUALTIY

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ABSTRACT

Freezing of boar semen is highly desirable; However, to date there have been no encouraging results for its use in the production of pigs for supply due to the damage that sperm suffer during the freezing-thawing process due to oxidative stress that impairs fertilizing characteristics such as motility, viability And acrosomal integrity (NAR). The objective of this work was to evaluate the effect of the addition of antioxidants (vitamin C, E and their combination) on the sperm quality (motility, viability and NAR) of frozen-thawed semen of boar in two types of straws. York boar semen was used. As a diluent, MR-A was used for semen transport. For the freezing the Westendorf method was used with some modifications in straws of 0.25 and 0.50 ml. Before freezing, vitamins C, E and C + E were added at concentrations of 1, 2 and 4 mg / ml. Thawing was performed seven days later. The best results were: With vitamin C at a concentration of 4 mg / ml in straws of 0.25 ml, 0%, 41% and 40% of motility, viability and NAR, respectively; In straws of 0.5 ml was 63%, 95% and 80% of motility, viability and NAR, respectively. With vitamin E at a concentration of 2 mg / ml, in straws of 0.25 ml, 40%, 66% and 73% of motility, viability and NAR, respectively, were obtained; In straws of 0.50 ml, 40%, 60% and 55% of motility, viability and NAR, respectively, were obtained. With the combination of vitamins C + E at a concentration of 1 mg / ml in straws of 0.25 ml, 0%, 47% and 60% of motility, viability and NAR, respectively, were obtained; In straws of 0.50ml, was obtained, 0%, 42% and 47% of motility, viability and NAR. In conclusion, the best results were with vitamins C and E, separately as antioxidants in straws of 0.50ml.

INTRODUCTION

Long-term conservation of semen is economically important and highly desirable for maintaining and conserving germplasm, preserving genetic diversity and improving reproductive efficiency of animals (Carpio *et al*., 2008). The swine industry looks for a way to optimize the productivity of boars for AI, however, the preservation of semen after ejaculation, particularly freezing, causes biochemical and functional changes to the spermatozoa, resulting in a reduction Of mobility and viability, with subsequent damage during transportation and fertilization, due to the generation of large quantities of reactive oxygen species (ROS) and consequently the formation of free radicals and the existence of oxidative damage related to poor conservation (Cordova *et al*., 2006; Roa *et al*., 2005). Antioxidants are molecules that prevent uncontrolled formation of free radicals or inhibit their reactions. They act as electron donors capable of avoiding an

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oxidation-reducing chain reaction and thus maintaining a pro-oxidant-antioxidant balance (Cordova *et al*., 2009). The objective of the present study was to evaluate the effect of the addition of antioxidants (vitamin C, E and their combination) on the sperm quality (motility, viability and acrosomal integrity (NAR) of frozen-thawed semen of boar.

MATERIAL AND METHODS

Boar ejaculate of the commercial breed York, from which Motility, Viability and Acrosomal Integrity (NAR) was evaluated. Semen freezing was carried out using the method described by Westendorf *et al*., 1975, with some modifications by Martín Rillo, 1989.

After analyzing each of the samples, the following concentrations of Vitamin C, E and their combination were used in the refrigerated and frozen semen extender (table1).

Table 1 Concentrations of Vitamin C, E and their combination

Vitamin C	Vitamin E	Combination of C and E
Tube 1: 0 mg/ml	Tube 1:0 mg/ml	Tube 1: 1 mg/ml + 1 mg/ml
Tube 1: 1 mg/ml + 1 mg/ml	Tube 2: 1 mg/ml	Tube 2: 2 mg/ml + 2 mg/ml
Tube 3: 2 mg/ml	Tube 3: 2 mg/ml	Tube 3: 4 mg/ml + 4 mg/ml
Tube 4: 4 mg/ml	Tube 4: 4 mg/ml	

For the refrigerated semen, which was already diluted in the MR-A diluent, it was divided into 11 test tubes, then the vitamins C, E and their combination were added in different concentrations (0, 1, 2 and 4 mg / ml) , Once the vitamins were added they were kept in refrigeration at 5 ° C. When the samples reached this temperature, the mobility, viability and NAR of each of the samples were analyzed, being carried out every 24 hours, until the samples lowered their sperm motility to 20%.

Before starting the freezing process for frozen semen, diluents A and B were prepared. To prepare diluent A, 11% lactose, 20% egg yolk and 80 ml of distilled water were used, the diluent Was centrifuged at 2000 rpm for 10 minutes in order to remove solids from the mixture and only the supernatant, which resulted from centrifugation, was used. For diluent B, lactose-egg yolk + 3% glycerol was used, this mixture no longer needed to be centrifuged.

Once diluents A and B were taken, 11 test tubes were prepared with the semen already diluted in MR-A. Subsequently all samples were equilibrated 90 minutes at room temperature (20-22 ° C). After this time they were brought to a temperature of 15 ° for 120 minutes. Once this was done, they were centrifuged at 2000 rpm / min for 10 minutes in order to obtain the sperm rich fraction, to which 3.5 ml of diluent a (lactose 11%, 20% egg yolk) At room temperature and subject to cooling temperature 5 ° C for about 3 hours. It was then diluted a second time in diluent B (lactose-yolk + 3% Glycerol) to 5 ml and subsequently added vitamins C, E and their combination at different concentrations (0, 1, 2, 3 and 4 mg / ml) to each of the tubes and homogenized. They were added in 2 equal proportions with difference of 15 minutes. At the end of the first portion, they were packed in 0.25 ml straws and the second portion was packed in 0.5 ml straws. And frozen. They were left there until the time of their thawing and subsequently, the motility, viability and NAR of each of the samples were evaluated.

RESULTS

Table 2 shows the results of the average of the different treatments (vitamin C, E and C + E) in their different concentrations in straws of .25 and .50 ml after thawing.

Table 2 Results obtained from the addition of vitamin C, E and C + E in different concentrations, in straws of .25 and .50 ml.

	FROZEN straws 0.25ml			FROZEN straws 0.50ml		
Treatment	Motility %	Viability %	NAR %	Motility %	Viability %	NAR %
Witness	47	45	45	47	45	45
With MRA diluent	85	83	82	85	83	82
C1*	0	38	55	0	49	40
C2*	0	38	57	0	46	36
C4*	0	41	40	63	95	80
E1**	36	73	85	37	64	56
E2**	40	66	73	40	60	55
E4**	39	62	67	38	58	53
C+E***	0	47	60	0	42	47
C+E***	0	44	38	0	38	35
C+E***	0	40	30	0	34	27

* Treatment with vitamin C at concentrations of 1, 2 and 4 mg / ml semen
** Treatment with vitamin E at concentrations of 1, 2 and 4 mg / ml semen
*** Treatment with the combination of vitamin C + E at concentrations of 1, 2 and 4 mg / ml semen.

DISCUSSION

Córdova *et al*. (2006) reported that oxidative stress is an imbalance between oxidants and cellular antioxidant mechanisms, therefore the use of vitamin E and C provides better conditions in the maintenance of sperm quality during the freezing process, Which is in agreement with the results obtained in the investigation regarding viability and acrosomal integrity, with the addition of these vitamins in their different concentrations (1, 2 and 4 mg / ml). However, as regards sperm motility, we did not obtain good results, as can be seen in Table 2, in the three different concentrations of vitamin C + E treatment in .25 and .50 ml straws, as in Treatment of vitamin C in the three concentrations in straws of .25 ml. This coincides with Membrillo *et al*., 2003, who reported that vitamin C has protective effects on the plasma membrane in equine spermatozoa, but does not aid in the improvement of sperm motility.

Cordova *et al*. (2005) points out that the storage form of frozen-thawed semen influences the temperature transfer and the viability of the sperm cells; Which is in agreement with the results obtained in this investigation when presenting differences between straws of 0.25 and 0.5 ml used in the freezing of the samples, as shown in table 2. A study by Carpio *et al*., (2008), in which frozen boar semen in 0.5 ml straws, reported percentages of motility and viability of 34.5% and 40.1% respectively, which is consistent with that reported by Córdova *et al*. , (2005). The results obtained in the 0.5 ml straws using treatments with vitamin C, E and their combination in concentrations 1, 2 and 4 mg / ml, similar results were observed in those straws with vitamin E in their different concentrations. In terms of viability we obtained better results, reporting 51.7% respectively; Obtaining the best

results of motility, viability and NAR with the addition of 4 mg / ml of vitamin C in straws of 0.50ml.

Another study conducted by Barrientos *et al*. (2009), where the effect of cryopreservation on the acrosomal integrity of boar spermatozoa was evaluated, found that the thawing process significantly reduced acrosomal integrity by 41% compared to the results (1, 2 and 4 mg / ml), the percentage of acrosomal integrity decreased by 20%, with the addition of vitamins C, E and their combination at different concentrations.

In conclusion, the best results were with vitamins C and E, separately as antioxidants in straws of 0.50ml.

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