



FRESH AND REFRIGERATED CONSERVATION OF BOAR SEMEN WITH ANTIOXIDANTS

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ABSTRACT

Oxidative stress affects the seminal conservation, mainly in pig sperm, since in these they are more susceptible to the effect of reactive oxygen species. The objective of this work was to assess the effect of the addition of vitamin C and E as antioxidants on motility, viability and acrosomal integrity (NAR) of boar semen preserved in fresh and refrigerated. A sample of boar ejaculate of the York commercial breed was used, which was diluted with a commercial diluent and vitamins C, E and C + E in concentrations of 1, 2 and 4 mg / ml, to later keep it fresh and in Refrigeration for 24 hours. Motility, viability and acrosomal integrity (NAR) were assessed. The best results were: with vitamin C in a concentration of 1 mg / ml, 46%, 76% and 95% of motility, viability and of NAR when it was kept fresh; when it was kept under refrigeration, 10%, 57% 55% motility, viability and NAR were obtained, respectively. With vitamin E at a concentration of 2mg / ml stored fresh, 66%, 93% and 80% of motility, viability and NAR were obtained, respectively; preserved in refrigeration, 73%, 63% and 68% of NAR were obtained, respectively. When the combination of vitamins C + E was used at a concentration of 2 mg / ml stored fresh, 13%, 85% and 82% of motility, viability and NAR were obtained. When the same concentration of vitamins E + C was used in refrigeration, 0%, 33% and 26% of motility, viability and NAR were obtained, respectively. In conclusion, the addition to the diluent of vitamins C, E and C + E as antioxidants, offer promising results for fresh and chilled conservation of boar semen.

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INTRODUCTION

Around the world, the use of Artificial Insemination (AI) in pigs has increased considerably due to the advantages they offer in the short, medium and long term such as: advance in genetic improvement, avoid direct contact of male- female, constantly evaluate sperm quality and thus ensure the success of fertilization, optimize the control of reproductive performance of the animal production unit, reduce the number of boars per female, which would be reflected in the reduction of maintenance costs of the same ones (Gadea, 2004). However, despite these achievements, the majority of AI services are performed using fresh or refrigerated semen doses, due to the low fertilization capacity of frozen sperm, due to the susceptibility of these to shock.

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Thermal, as a result of the characteristics of its plasma membrane (Roca *et al.*, 2005). The processes of refrigeration and freezing, mainly, produce physical and chemical alterations in the spermatid membrane, this due to the reduction of the temperature that is associated to the so-called oxidative stress; which has been defined as an imbalance between oxidants and cellular antioxidant mechanisms, which is induced by the generation of reactive oxygen species (ROS). In the pig, the sperm membrane has a high content of phospholipids linked to polyunsaturated fatty acids and low cholesterol content, characteristics that make it vulnerable to suffer oxidative stress; In addition, the spermatozoa of this species have a small amount of cytoplasm that limits the antioxidant action of the repair enzyme systems (Membrillo *et al.*, 2003). One promising area of study is the possible pretreatment against the processes of peroxidation of the sperm in the semen dilution medium to protect or preserve the integrity of the sperm membrane (Membrillo *et al.*, 2003).

The objective of this work was to assess the effect of the addition to the diluent of vitamin C, E and its combination as antioxidants on motility, viability and acrosomal integrity in boar semen preserved in fresh and refrigerated.

MATERIAL AND METHODS

A sample of the boar ejaculate of the York commercial breed was used, Motility, Feasibility and NAR were evaluated. S used concentrations of vitamins C, E and their combination (C + E) as antioxidants in the diluent in boar semen preserved in fresh and refrigerated:

Vitamin C	Vitamin E	Combination C y 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	

The conservation of fresh semen and refrigeration was carried out based on the method described by Westendorf *et al.* (1975), with some modifications described by Martín Rillo (1989). In the case of fresh semen, it was kept in 80 ml of commercial diluent MRA, 11 test tubes were prepared, with diluted semen, to which were added vitamins C, E and their combination in their different concentrations (0, 1, 2 and 4 mg / ml) and they were maintained at room temperature, later the mobility, viability and NAR of each of the samples was analyzed every 24 hours. The semen refrigerated and diluted in MR-A, was distributed in 11 test tubes, later the vitamins C, E and its combination in different concentrations (0, 1, 2 and 4 mg / ml) were added, they were kept in refrigeration 5°C. When the samples reached this temperature, mobility, viability and NAR were assessed every 24 hours, until the samples showed sperm motility of 20%.

RESULTS

Table 1 shows the results of the evaluation of the initial semen quality and after 4 hours. These results did not show a great difference between the ejaculates in terms of motility, viability and acrosomal integrity at the beginning.

Table 1 Results obtained from the semen samples before the addition of vitamins C, E and their combination.

Sample	Motility %	Viability %	NAR %
Testigo*	93	94	95
Testigo**	47	45	45

*At the beginning.
** At 4 hours.

In Table 2, you can see the results of the samples evaluated in their motility, viability and NAR, both fresh and refrigerated semen after the addition of vitamins C, E and C + E.

Table 2 Average results of fresh and refrigerated semen

Treatment	FRESH			REFRIGERATED		
	Motility	Viability	NAR	Motility	Viability	NAR
Witness	47	45	45	47	45	45
C1*	46	76	95	10	57	55
C2*	20	86	95	4	47	52
C4*	0	86	85	0	51	56
E1**	66	75	95	29	59	73
E2**	66	93	80	73	63	68
E4**	63	95	80	39	63	72
C+E***	11	93	80	3	50	52
C+E***	13	85	82	0	33	26
C+E***	0	73	70	0	18	16

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

DISCUSSION

Alemán (2005) indicated that refrigerated semen can be stored at a temperature of 15°C. For this it is necessary to use a diluent with cryoprotective substances being the most used egg yolk and milk. Conservation at 15°C is the most used in pig production units. Good results have been obtained experimentally, with the use of MR-A which allows to maintain viable the spermatic material at 17°C for about 5 days, compared to the results obtained in the present investigation we affirm that if it is important to use a diluent for keep the semen in refrigeration, but with the addition of vitamins C, E and their combinations, in different concentrations (1, 2 and 4 mg / ml) favorable results were found as shown in table 2, in the addition of vitamin E in its three concentrations. The boar sperm suffers damage caused by the process of freezing-thawing; Barrientos *et al.*, (2009) noted that only 50% of sperm survive this process, reporting results of 46.4% in acrosomal integrity and 34.9% in a study conducted in the porcine species. In this investigation, higher percentages were found than those presented by these authors, due to the influence obtained when adding vitamin C, E and its combination at different concentrations (1, 2 and 4 mg / ml) in freezing thinners, such as is shown in table 2, in almost all combinations (except in the combination of vitamin C + E at a concentration of 2 and 4 mg / ml). In conclusion, the addition of vitamins C, E and their combination as antioxidants used in boar semen maintain sperm quality when conserving semen in fresh and refrigerated; However, it is necessary to continue looking for a good technique to dilute these vitamins and in this way be able to use all their antioxidant benefits.

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