ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 03, pp.47599-47600, March, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EFFECT OF ADDITION OF ANTIOXIDANTS IN THE FREEZING OF BOAR SEMEN ON THE MOTILITY AND VIABILITY OF SPERM

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ARTICLE INFO

Article History:
Received 08th December, 2016
Received in revised form
15th January, 2017
Accepted 15th February, 2017
Published online 31st March, 2017

Key words:
Antioxidant,
Vitamin C,
Vitamin E,
Semen Frozen-Thawed Boar.

ABSTRACT

The aim of this study was to assess the effect on the use of vitamin C, E and C + E as antioxidants on sperm quality of frozen-thawed semen boar semen Yorkshire breed, which was divided into three treatments with Vitamin C, E and combined (C + E) at different concentrations (0, 2, 4 and 6 mg / ml) was used. Motility and viability was assessed before and after freezing the semen, semen freezing was described by Westendorf based with some modifications. After thawing with vitamin C motility of 80% and viability of 75%, with vitamin E motility was obtained was 85% and a viability of 89% and vitamin C + E motility was 85% and 88% viability. Concentration of 4 mg / ml in the control treatment vitamin C was 81% and 85% viability, with E motility was 80% and 85% viability. With vitamins C + E motility was 86% and 88% viability before freezing. After thawing, motility vitamin C was 80% and 80% viability; with E motility was 80% and 83% viability; with C + E vitamins in the witness vitamin C motility was 83% and the viability of 85%: In the concentration at 6 mg / ml the witness with vitamin C motility was 81% and viability of 80% ; with E motility was 87% and the viability of 80% and vitamins C + E motility was 85% and the viability of 88%: After thawing, the C motility was 83% and 79% viability, with E motility was 85% and 83% viability with vitamins C + E motility was 87% and 88% viability. In conclusion, the addition of vitamins in the spermatic conservation help keep sperm motility and viability.

Citation: Alejandro Córdova Izquiedo, Adrian Emmanuel Iglesias Reyes, Román Espinosa Cervantes, Juan Eulogio Guerra Liera, Jorge Fabio Insunza Castro, Abel Edmundo Villa Mancera, Maximino Méndez Mendoza, Rubén Huerta Crispín, María de Lourdes Juárez Mosqueda, Armando Gómez Vázquez, Pedro Sánchez Aparicio, Jaime Olivares Pérez, Blanca Estela Rodriguez Denis and Silvia Denise Peña Betancurt. 2017. "Effect of addition of antioxidants in the freezing of boar semen on the motility and viability of sperm", *International Journal of Current Research*, 9, (03), 47599-47600.

INTRODUCTION

The spermatozoon is a highly specialized cell with the ability to actively move to fertilize the egg, but damage to the sperm plasma membrane leads to an irreversible loss in their duties. The integrity of the sperm cells may undergo many risks, and one of the most damaging is the oxidative stress induced by reactive oxygen species (ROS) (Vallorani *et al*., 2010).

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Plasma membranes of boar spermatozoa contain a relatively high proportion of polyunsaturated fatty acids that undergo lipid peroxidation after cryopreservation. Lipid peroxidation has been correlated with exposure of sperm from free radicals (reactive oxygen species, ROS). Excessive ROS formation during the cryopreservation has been associated with a decrease in the quality and fertility of sperm after thawing (Buranaamnuay *et al*., 2011). During the production of frozen pig semen, sperm cells are exposed to a number of potential hazards. These factors include dilution, incubation, staining, pressure, centrifugation and freeze-thaw process. Some reports have shown that cryo-injury induced by freeze-thawing can be

minimized by optimizing the cooling rate and cryoprotectant. A number of cryoprotectants and freezing-thawing protocols have been tested for boar semen (Jiang *et al*., 2007). In recent years, research on the application of antioxidants to improve pig sperm cryopreservation and improve semen quality after thawing has been extensive. There are two types of antioxidants: enzymatic and nonenzymatic antioxidants antioxidants. Both types can neutralize excess ROS and prevent cell structure (Zhang *et al*., 2012) damage. So the aim of this study is to assess the effect on the use of vitamin C, E and C + E as antioxidants on sperm quality of frozen-thawed boar semen.

MATERIALS AND METHODS

Semen of a Yorkshire pig race with manual technique was obtained was diluted with MRA to be transported and evaluated. Semen freezing took based on the method described by Westendorf *et al*., (1975), with some modifications (Martin Rillo, 1989). For cooling at 5 ° C it was dissolved in the extender A (Table 1) and added to each of the test tubes, vitamins C, E and C + E in doses of 2, 4 and 6 mg / ml, He was placed in a container with water and put in the refrigerator for 3 hrs. Once completed time for freezing, glycerol diluent (extender B) (Table 1) was added in two parts with an interval of 15 minutes each part always having a constant temperature of 5 ° C.

Table 1. Composition of the extender A and B.

Component	Extender A	Extender B
TRIS	5.65g	5.65g
Citric acid	3.80g	3.80g
Dextrose	27.5g	27.5g
Antibiotic	2g	2g
Distilled water	1000ml	1000ml
Glycerol	-	20%*
Yolk	20%*	20%*

* Indicates 20% of total volume.

Table 2. Evaluation of semen concentration of 2 mg / ml

	Extender A		Extender B	
Treatment	Motility (%)	Viability (%)	Motility (%)	Viability (%)
Control	75	80	80	75
C	78	83	83	81
E	80	84	85	89
C+E	82	83	85	88

The extended semen was packaged in straws 0.25 and 0.5 at a concentration of 1.2 million sperm per straw. Then straws were placed in freezing with liquid nitrogen (-196°C). Finally pulled straws, motility and viability of frozen sperm was evaluated.

RESULTS

Tables 2, 3 and 4 the results obtained at concentrations of 0, 2, 4 and 6 mg / ml diluent A and B, adding vitamins C, E and C + E are shown.

Table 3. Evaluation of semen concentration of 4 mg / ml

	Extender A		Extender B	
Treatment	Motility (%)	Viability (%)	Motility (%)	Viability (%)
Control	75	80	80	75
C	81	85	80	80
E	80	85	80	83
C+E	86	88	83	85

Table 4. Evaluation of semen concentration of 6 mg / ml

	Extender A		Extender B	
Treatment	Motility (%)	Viability (%)	Motility (%)	Viability (%)
Control	75	80	80	75
C	81	80	83	79
E	87	80	85	83
C+E	85	88	87	88

DISCUSSION

As shown in Table 2, 3 and 4 the three treatments a remarkable improvement over the control treatment are appreciated. However, the treatment of vitamins C + E (combined) proved to be superior to others in the 3 concentrations. In the treatment with a concentration of 6 mg / ml of vitamin E (Table 4), a significant difference was seen in motility; in the same vein treatment C + E proves to be more favorable. Observing the three concentrations, shows that in the concentration of 2 mg / ml (Table 2) there is considerable variation; instead by raising this, treatment C + E starts to make a significant difference. It is very important to note that the three treatments, the most effective was the one containing the combination of vitamin C + E B with the diluent, at a concentration of 6 mg / ml. This is because as mentioned Zhang *et al*., 2012 ascorbic acid (vitamin C) is a ROS scavenger, water soluble low toxicity and high power. As an antioxidant chain rupture, you can capture and remove free radicals during the chain reaction which propagation of peroxidation process and α-Tocopherol (vitamin E) is stopped, the soluble antioxidant predominant lipids cells animals can break the covalent bonds formed between the ROS side chains of fatty acids in membrane lipids, thus protecting the membrane components way without influencing the generation of ROS. In conclusion, the addition of vitamins in the spermatic conservation help reduce peroxidation.

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