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Addition of Antioxidants in the Diluter for the Conservation in Fresh of Boar Semen

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Abstract: The objective of this research was to value the effect of the addition of vitamins C, E and the combination C+E in diluted fresh boar semen on motility and acrosome integrity (NAR). Were used ejaculated coming from a boar of the race Pietrain with two year-old age three months, with weight of 350 kg. were carried out three experiments. The first with vitamin C, the second with vitamin E and the third with vitamin C+E; with concentration of 5 mg mL^{-1} of diluter of each vitamin for each experiment; a witness, only with diluted semen, were conserved at 18°C in a period of seven days, every other day the motility and acrosome integrity were evaluated. They were carried out 3 repetitions for experiment and the obtained results went for spermatic motility to the seven days of conservation, tha treatment with the vitamin E (46.6%), with vitamin C (0%) and with vitamins C+E (1.08), comparison with the witness (64.1%). For NAR, the following results were had, with Vitamin E (62%), with vitamin C (57.6%) and with vitamins C+E (60.6%), in comparison with the witness (70.8%). When carrying out the variance analysis, they were differences statistically significant ($p<0.05$) among the treatment with vitamin C and C+E on the motility and NAR in comparison with the witness. In conclusion, the effect that was obtained on the motility and NAR, when vitamins C and C+E were used to a concentration of 5 mg mL^{-1} of diluter, it requires of more investigation in the area of the conservation of the hog semen in fresh, using vitamins E, C and their combination, vitamins E+C.

Key words: Boar semen, conservation in fresh, motility, acrosome integrity, antioxidants

INTRODUCTION

The possibility to store the semen for a while lingering, without losing their capacity fecundate gave origin to the beginning of a new era in the development of the Artificial Insemination (IA) in the units of Swinish Animal Production as a technique of wide application in the entire world, although the use grade in the diverse countries is very variable. In Europe is considered that the application of the IA is very high arriving to rates superiors of 80%, while in United States the application of this biotechnology is of 50%. Mexico has a reduced application although, in the last years the rate has been increased in 30% (Gadea, 2003; Córdova *et al*., 2001; Córdova *et al*., 2006).

To have good results in the IA, it is of supreme importance to conserve in good state the spermatic cells, the viability of the sperms of swinish in the one ejaculated is limited to only some few hours, this makes necessary the dilution that is the total elimination of the seminal plasm and the subsequent conservation of the sperms in a diluter with characteristic physiologic, biochemical and biophysical that allows its conservation (Johnson *et al*., 2000; Córdova *et al*., 2001).

Gadea (2003) indicated that it is necessary to continue working with diluters dedicated to improve the conservation of the swinish semen, since the study that you/they play the diluters in the seminal conservation is indispensable. This can explain to you with more easiness, the sperms are by itself in the seminal plasm that gives the necessary nutrients to maintain a high necessary metabolic activity for the process of spermatic transport through the reproductive tract of the female, the seminal plasm it doesn't allow there to be a durable conservation of the semen.

The survival of the cells takes place under aerobic conditions the Species you Reactivate of Oxygen (ROS) they are part of the normal metabolic activity. The spermatic cells and the seminal plasm of the mammals are not the exception and these considerable quantities of ROS that will attack the rich plasmatic membrane specifically in phospholipids causing the phenomenon of

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lipidperoxidation take place or also well-known as lipidperoxidation that is caused by the ROS that induce to the oxidation of the lipids causing the aging and cellular death (Cerolini *et al*., 2000; Membrillo *et al*., 2003; Gupta and Prasad, 2004; Brouwers *et al*., 2005).

To weigh that the sperms are protected by a system of antioxidant natural, these they can be overcome, in situations that the excessive formation of the ROS causes oxidation and for such a reason loss of their physiologic characteristics that you/they allow him to carry out the fecundation with success (Funahashi and Sano, 2005; Córdova *et al*., 2006). In spite of the advances in the conservation of the germinal material, the physiologic differences of the spermatic cells among the species and even the man, still represents a problem without solving (Córdova *et al*., 2004) making emphasis to this, the sperms of the species of mammals, the hog is the most vulnerable to the lipidperoxidation, this associated with the high relationship of fatty acids poliinsaturate-saturated in the phospholipids and a contained first floor of cholesterol in the plasmatic membrane. It is known that the seminal plasm of the hog is not precisely rich in substances with anti-rust capacity (Cerolini *et al*., 2001; Roca *et al*., 2002).

With base to the above-mentioned, an alternative is the pre-treatment against the processes of peroxidation of the sperms, this pre-treatment is to add to the antioxidant diluter to protect to the sperms of the abrasive changes that for mere biological mechanism during the conservation. The spermatic cells, conserved in frozen state are more exposed to damages for crash to the cold, at the moment for the IA they are continued using more the seminal doses conserved in state fresh-refrigerated to temperature from 15 to 20°C for the disadvantages that it presents the freezing and unfreezing (Huo *et al*., 2002).

Recent investigations made by Upreti *et al*. (1997), Johnson *et al*. (2000) and Mara *et al*. (2005) they indicated that the diluters could contain antioxidant that avoid the imbalance in the formation of the ROS and the action of the anti-rust ones to slow the spermatic lipidperoxidation. The sperms when being manipulated before and during their conservation they are exposed to the oxygen and the radiation of the light what induces to the formation of the ROS, being irreversibly to damages in what concerns to the motility and integrity of the membrane, acrosome and DNA (Hansen *et al*., 2005).

An antioxidant with biological funtion has been defined as a substance that diminishes or it avoids the oxidation of the substrate being an agent more potent reducer. Diverse protocols of seminal conservation highlight to the vitamins C and E as powerful antioxidants.

The vitamin E, is it known that it is an essential nutrient for the reproductive acting from 1922, inside this term it derives the group of the tocofherols and the-tocofherol the most abundant is in the nature having a biological and very effective high activity acting as cleaner of free radicals, preventing the lipidperoxidation of the cells (Flohé and Traber, 1999; Sarlos *et al*., 2002; Argawal, 2004; Breininger *et al*., 2005).

Studies carried out *in vitro* by Roca *et al*. (2002) showed that the action of the vitamin E envelope the motility of the semen varies depending on the spermatic physiology, level of antioxidants contents in the seminal plasm and the susceptibility of lipidperoxidation of the membrane of each species. In other investigations (Upreti *et al*., 1997; Sarlos *et al*., 2002) mentioned that the vitamin E when was added to the ram semen it was harmful since it showed a reduction in the motility, in studs had very reduced effect and inclusive none; as well as in male chevron, on the contrary (Cerolini *et al*., 2000) demonstrated in vitro that the vitamin E had a protective effect on the integrity of the membrane and it improved motility when was added to the fresh boar semen.

Breininger *et al*. (2005) did they point out that using vitamin E (α-tocopherol), as treatment antioxidant attaché to the semen cryopreservate of bovine, did it prevent of the oxidative damage, also with fresh boar semen maintained to a temperature of 19°C.

The ascorbic acid (vitamin C) it is a substance antioxidant hydrosoluble that is present in the flowing epydimiaries and seminal plasm of many species. The ascorbic acid can play a significant paper as protective of the sperms in the excessive formation of the ROS and maintaining the genetic integrity of the spermatic cells, preventing from the oxidative damage to the DNA (Sönmez and Demirci, 2004).

Donoghue and Donoghue (1997) indicated that the group of antioxidant hydrosolubles in this case the vitamin C, reduces the peroxidation outside of the cells, but that has very little effect in the membrane and inside the cell, the opposite of the effect of the antioxidants lipidsolubles like the vitamin E that minimize the damage to the plasmatic membrane.

The investigations that are had about the use of the vitamin C like antioxidant in semen under conditions *in vitro* of different species, including the man has had very not very favorable results, because it is a highly sour vitamin causing reduction in pH and that if doesn't administer himself to the appropriate doses it acts as pro-oxidizer inside the cells (Carr and Frei, 1996; Aurich *et al*., 1997; Sánchez *et al*., 1997; Ball *et al*., 2001; Rivadeneyra *et al*., 2001; Argawal, 2004; Sönmez and Demirci, 2004).

With the references before mentioned of the antioxidants properties of the vitamins C and E, did explain that the vitamin C the main one is antioxidant in the plasm and inside the cell, when donating electrons to the radical tocopheroxil of the vitamin E rusty and that this way it recycles the antioxidant function of the α-tocofherol helping to protect the lipdic membrane of the peroxidation. As well as the vitamin C can act as pro-oxidizer, the vitamin E also, since it inhibits or it facilitates the peroxidation lipidic of the lipidproteins of low density. The activity pro-oxidizer of the α-is tocofherol prevented by the ascorbic acid, for that that the vitamin E can it only be effective in combination with the vitamin C. The combination of the vitamin E, an antioxidant lipidphyilic, with vitamin C, desintoxic an antioxidant hydrophyilic the lipid of the peroxides.

The objective of this research was to value the effect of the addition of vitamins C, E and the combination C+E in the diluter for the conservation of the diluted fresh boar semen on the motility and acrosome integrity (NAR).

MATERIALS AND METHODS

Animals and study place: The seminal samples were obtained of the same hog race Pietrain, 2 years old 3 months with a weight of 350 kg coming from a Center of Swinish Artificial Insemination located in the Delegation Tlahuac, D.F.

Collection and preparation of the semen: The collection of semen was carried out every ten days by means of the method of artificial vagina, once obtained the seminal samples were diluted with a diluter it marks commercial, MR-A of long duration, later on they were taken to the laboratory to value them and to begin the treatments with the vitamins E and C.

Valuation of the semen: The seminal valuation was carried out after the collection and dilution of the semen to estimate the parameters (spermatic motility and acrosome integrity), before beginning the experiments with the vitamins, every other day the seminal samples were valued and changes were observed in the motility and acrosome integrity.

Spermatic motility: Sperm motility was evaluate using an optic m icroscope to 40 increases by placing a drop of the sample, micrsocope slide and covering with a cover slip. The results of motility were expressed as percentage of cells spermatic motives of 0-100 (Córdova *et al*., 2004).

Acrosome integrity: The percentage of normal acrosomes was valued by means of the technique of tint eosine-nigrosine described by García *et al*. (1994).

The tint eosine-nigrosine got ready in the following way:
Eosine 1%
Nigrosine 4%
Dilution in 200 mL of distilled water

The evaluation acrosome integrity was done by examining with a phase contrast microscope, at 100X magnification. A minimum of 100 acrosomes were examinated per sample. Damage to the acrosome integrity was classified by the scoring system report by Pursel *et al*. (1992).

Preparation of the samples of semen with the vitamins C, E and E+C: The experiments were carried out for triplicate, conserving the doses in a period of 9 days to ambient temperature of 18°C and with evaluation of the spermatic motility and acrosome integrity every other day.

Experiment 1
Witness: Samples of 50 mL of fresh semen diluted without adding any vitamin.

Experiment 2: Samples of 50 mL of diluted fresh semen of hog and addition of 5 mg mL^{-1} of vitamin C of commercial mark "Redoxon C" of laboratories Roche.

Experiment 3: Samples of 50 mL of diluted fresh semen of hog and the addition of 5 mg mL^{-1} of vitamin E commercial product of the laboratory of Paris Pharmacy.

Experiment 4: Samples of 50 mL of diluted fresh semen of hog adding the combination of 5 mg mL^{-1} of vitamin C+ 5 mg mL^{-1} of vitamin E.

Statistical analysis: The analysis of the results was carried out, by means of the Analysis of Variance (JMP version Programs 3.1.2. 1997. SAS Institute.Inc).

RESULTS

The results obtained in the different treatments, are presented in the Table 1 and 2.

Table 1: Effect of the vitamins E, C and C+E added on the spermatic motility

		Percentage of motility			
Treatment	Repetitions	Day 1	Day 3	Day 5	Day 7
Vitamin E	3	66.6	61.6	51.6	46.6
Vitamin C	3	0	0	0	0
Vitamins C+E	3	1.08	0	0	0
Witness	3	73.3	68.3	65.2	64.1

Table 2: Effect of the vitamins E, C and C+E added on the acrosome integrity

		Percentage acrosome integrity			
Treatment	Repetitions	Day 1	Day 3	Day 5	Day 7
Vitamin E	3	73	70	67	62
Vitamin C	3	68.3	65.6	61	57.6
Vitamins C+E	3	71.3	67	63.6	60.6
Witness	3	78.2	75.1	72.3	70.8

DISCUSSION

The addition of antioxidant, in the diluter provides protection for the spermatic cells, improving the motility and the acrosome integrity during the conservation.

You can consider that the seminal doses during their conservation are preserved against the processes of peroxidation of the sperms and that the means of dilution contains antioxidant to protect and to conserve the integrity of the membrane, acrosome and motility, due to the normal metabolic activity of the ROS in the seminal plasm. It is known that the membrane of the spermatic cells of hog is very susceptibility to the lipidper-oxidation, because the membrane is composed by a high content of poliinsaturate-saturated fatty acids (Funahashi and Sano, 2005).

In this research, vitamins C was used, E and the combination of vitamins C+E with dose of 5 mg mL^{-1} in each treatment. Their effect was valued about the motility and acrosome integrity in fresh boar semen conserved to temperature of 18°C.

The effect of the addition of 5 mg mL^{-1} of vitamins, C and C+E in diluted fresh boar semen on the percentage of motility, the two treatments compared with the witness, based on the variance analysis significant results was obtained ($p<0.05$) (Table 1). Where the treatment with the vitamin C about the spermatic motility at the 24 h showed 0% compared with the witness that until the day 7 of conservation revealed 64.1% of motility. Recent studies demonstrated that the vitamin addition C in dose of more than 2 mg mL^{-1} in diluted semen of ram reduced the spermatic motility during their conservation in liquid state (Sönmez *et al*., 2004). Inclusive in humans high vitamin dose C added to the semen for its conservation lowers the motility progressively until 10% (Rivadeneyra *et al*., 2001) this can be related with the decrease of the pH caused for that the vitamin C that is strongly sour, pH 2 and such a low pH can induce reduction irreversibly in the motility of the sperms (Aurich *et al*., 1997; Sánchez *et al*., 1997).

For the treatment with vitamin E, the results showed a decrease from the motility to day 7 with 46.6% regarding the witness (Table 1). The addition of 5 mg mL^{-1} of vitamin E to the fresh boar semen conserved at 18°C, in this work it maintained a percentage of low spermatic motility in comparison with the witness, therefore, the vitamin E in contrast with the addition of 5 mg mL^{-1} of vitamin C and 5 mg mL^{-1} of vitamin C + E to the diluted semen it was not harmful, due to their antioxidant power, as Donogue *et al*. (1997) mentioned when was added vitamin E to the turkey semen diluted for their conservation; Upreti *et al*. (1997) carried out an experiment adding vitamin E to the ram semen and the 24 h of conservation to temperature 15°C declined the motility; Sarlos *et al*. (2002), they indicated that the motility diminished along 9 days of conservation when was added vitamin E to the diluted semen of ram. In human it was indicated that in a same way, high dose, 1 at 3 mg mL^{-1} of vitamin E added to the semen it didn't improve the motility (Argawal *et al*., 2004).

In accordance with that pointed out by Breininger *et al*. (2005) the effect of the vitamin E (α-tocofherol) added to the hog semen it can vary with the concentration, does high dose instead of working as an antioxidant one act as stimulative of the oxidation (pro-oxidizer).

The results of the treatment with vitamins C+E showed 1.08% of motility starting from 24 h in comparison with the witness (Table 1); however, it is observed that in that time, it affected in 5% the acrosome integrity like it is shown in the Table 2. Has been indicated that when they combine the vitamins E and C inhibits the peroxidation of the cells, so that the activity pro-oxidizer of the vitamin E it is prevented by the vitamin C, for that the vitamin E alone it can be effective in combination with the vitamin C. nevertheless, in this study, the results didn't favor the motility when it was added to the diluted semen of hog the combination of 5 mg mL^{-1} of vitamin C and 5 mg mL^{-1} of vitamin E, this can be related to the high dose of vitamin C, was already mentioned in more quantities, more than 2 mg mL^{-1} added to the semen, its strong acidity, causes a decrease in the pH of the cells diminishing the spermatic motility considerably (Aurich *et al*., 1997; Sánchez *et al*., 1997).

The results that they were obtained in this research with the treatments about the acrosome integrity they are presented in the Table 2. Where the treatment of 5 mg mL^{-1} of vitamin C showed 57.6% of integrity acrosome in comparison with the witness that a day 7 of conservation, which presented 70.8%.

The treatment with vitamin E, had a minimum difference in the percentage of integrity acrosome of 68% in comparison with the witness that showed 72% acrosome integrity a day 7 (Table 2). The results of the percentage of integrity acrosome of the different treatments are diminished according to the time of

conservation regarding the witness, it is considered that the vitamin E the same as the vitamin C in a concentration of 5 mg mL^{-1}, didn't improve the results. The effect of the vitamin E envelope the motility and integrity acrosome can vary depending the concentration, when administers himself high concentration the vitamins E it acts as stimulative of the cellular oxidation (Ball *et al*., 2001; Breininger *et al*., 2005). The discrepancy between the obtained results of the motility and acrosome integrity with the different treatments is subject to the physiology and composition of the spermatic membrane, depending on the semen of each species, as Cerolini *et al*. (2001) and Roca *et al*. (2002). Consequently, it would be necessary to adapt the doses to lower quantities of the one used, mainly with regard to the vitamin C.

CONCLUSION

The use of the vitamins C and E they provide of antioxidant benefit, it is necessary to use them in appropriate quantities. Therefore, addition of vitamins C and C+E to a concentration of 5 mg mL^{-1} in diluted boar semen had a harmful effect on spermatic motility; however, the administration of these same vitamins regarding the acrosome integrity, they didn't show accented damage. On the other hand, the use of 5 mg mL^{-1} of vitamin E, although it didn't show significant improvement, neither it showed damage significant as the vitamins C and C+E, about the spermatic motility and as for the protective effect of the acrosome integrity, the vitamin E it didn't improve neither it maintained this parameter significantly, in comparison with the witness; it is necessary to continue working in the topic of the antioxidant ones in the conservation of boar semen.

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