Effect of lycopene on fowl sperm characteristics during in vitro storage

Einfluss von Lycopen auf die Eigenschaften von Geflügelsperma während der in vitro Lagerung

Maria Grazia Mangiagalli, S. P. Marelli and L. Guidobono Cavalchini


Introduction

Avian semen preservation during liquid storage has been object of investigations by many authors (WISHT, 1989; THURSTON, 1995; SEXTON, 1988a,b; CHRISTENSEN, 1995), particularly as regard to turkey semen, as the commercial production of turkey relies on artificial insemination. Chicken and turkey sperm contain high amounts of polyunsaturated fatty acids (PUFA) (RAVIE and LAKE, 1985; CEROLINI et al., 1997) and spontaneous peroxidation occurs during in vitro storage in both species (CECIL and BAKST, 1993). For this reason, the addition antioxidants to the diluent take a special interest in the development of commercial extenders. The avian semen dillents are composed of sugars, proteins and buffers, and many studies have been conducted on this subject by several authors (VAN WAMBEE 1972; LAKE and RAVIE, 1979, 1981; SEXTON et al., 1980; BOOTWALLA and PROMAN, 1988).

Natural antioxidants as vitamin E (Vit. E) are present in poultry semen (SURAI, 1981, 1989, 1999; SURAI and IONOV, 1992). The protective effect of vit. E included into the extender is well known (BLESBOIS et al., 1993; SURAI et al., 1997).

Vitamin E, butylated hydroxytoluene (BHT), Tempo (a lipid- and water-soluble commercial antioxidant; Sigma Chemical Co., St. Louis, MO) maintains turkey sperm membrane integrity during 48 h in vitro storage at T=5°C (DONOGHUE and DONOGHUE, 1997). A protective effect of vit. E on sperm membrane PUFA and membrane integrity, that many studies confirm their correlation with the motility and viability sperm parameters and with fertilizing capacity, was found in turkey spermatozoa at room temperature (MALDJIAN et al., 1998). An increasing interest is developing for supplementation of natural antioxidants commercial dillents to improve stored semen characteristics.

Carotenoids are considered an important group of natural antioxidants, their antioxidant activities have been the object of many studies (KRINSKY, 1989, 1998; EDGE et al., 1997; BAST et al., 1998) and their protective function in association with other antioxidants has been described in avian tissues (SURAI, 2002). Lycopene is a carotenoid found in ripe fruit, especially in tomatoes: one kg of fresh ripe to-
tatoes contains 0.02 g of lycopene (MERCK INDEX, 1996). The positive properties of lycopene are object of recent investigations (RAO and LEI, 2002; HARTAL and DANZING, 2003; COX et al., 2003). Several dietary studies have shown that a high intake of lycopene is associated with decreasing risk of prostate and cardiovascular diseases, lung and digestive cancers (TAPIERO et al., 2004; Wu et al., 2004). Diets rich in lycopene and other antioxidants are significantly correlated with antioxidants plasma level and contribute to antioxidant defence (SVILAAS et al., 2004; TYSSANDIER et al., 2004).

Some investigations demonstrated antioxidant activity of topicaly applied lycopene as prevention of cutaneous damage by free radicals; its antioxidant ability is probably due to its high reductive power (ANDREASSI et al., 2004). The aim of this research was to investigate the effect of lycopene supplementation on fowl semen characteristics during in vitro storage.

Materials and Methods

Birds and semen collection

Thirteen broiler breeders, Ross 508 grand parents, were kept in single cages under standard controlled environment (15L: 9D; T=19÷21°C) and fed with standard commercial diet for breeders (11.5 MJ ME/Kg CP 15.5%, CF 3.7%, Met 0.39%, Lys 0.71%; Ca 2.8%, P 0.35%, Na 0.16%, K 0.75%; Cu 10 mg-1Kg, Fe 60 mg -1Kg, Mn 60 mg-1Kg, Zn 100 mg-1Kg, Se 0.20 mg-1Kg) according to Ross broiler breeder management guide (145 g/d). The roosters were during the 36th and 43rd week of age in the trial (6 weeks) and semen was collected twice a week by abdominal massage (LAKE and STEWART, 1978).

In vitro storage conditions

Once a week semen samples were pooled and semen concentration was counted by spectrophotometer (\(\lambda=535\) nm, UV-1601 Shimadzu Kyoto, Japan). The pools were divided into 4 aliquots; each aliquot was diluted to 2x10^9 spermatozoa (spz)/mL in Lake's diluent (LAKE and RAVIE, 1979), and lycopene as water-soluble powder was added to Lake extender (Lycopene 10% WS, Roche Vitamins Ltd., Basel, Switzerland: sucrose 330, corn starch 250, fish gelatin 200, lycopene 100, corn oil 55, ascorbyl palmitate 50, dl-alpha-tocopherol 15 (mg x g^-1)). The samples were incubated in a water bath (T=13÷14°C) with gentle shaking for 24 h.
Antioxidant treatments

Fresh semen pool was evaluated at 0 h time and 500 µL of pool for treatment was diluted. The control was the extended semen with Lake; the experimental treatments were extended semen with Lake supplemented with lycopene in different concentrations: 500 µg/mL (500 L), 250 µg/mL (250 L) and 100 µg/mL (100 L). The working solutions for Lake with lycopene were prepared by serial dilutions of a stock solution (1000 ppm; pH = 7.13).

The sperm quality traits motility, forward progressive motility and viability were evaluated at 1 h, 6 h and 24 h. The sperm motility was evaluated subjectively by a phase contrast microscope (20x) at room temperature (n = 3 replicate observations for any time storage): 10 µL of extended semen was placed on Makler Chamber, a counting chamber specifically designed for semen analysis (MAKLER, 1980), and assessed as percentage of motile cells of observed total cells. The forward progressive motility (FP) was expressed as score by a scale from 1 to 4 (1 = no forward movement, 2 = slow linear movement, 3 = good linear movement, 4 = rapid linear forward progression). To assess sperm viability parameter the nigrosin/eosin (N/E) staining procedure according to BAKST and CECEL, (1997) was used. About five hundred spermatozoa in each preparation were examined by microscope (32x) and the viability parameter was expressed as percentage of live cells of total number of counted spermatozoa; besides, sperm morphological anomalies were observed.

Statistical Analysis

The data sets were analyzed by ANOVA using the method of least square and General Linear Models Procedure of SAS® (SAS, 1999).

Results

The fresh semen motility average, evaluated at 0 h time, was 77.5% and the FP score value was 3 for all replicate observations (Table 1). No significant difference was observed between lycopene treatments and control for the observations (Table 1). No significant difference was observed total cells. The forward progressive motility (FP) was expressed as score by a scale from 1 to 4 (1 = no forward movement, 2 = slow linear movement, 3 = good linear movement, 4 = rapid linear forward progression). To assess sperm viability parameter the nigrosin/eosin (N/E) staining procedure according to BAKST and CECEL, (1997) was used. About five hundred spermatozoa in each preparation were examined by microscope (32x) and the viability parameter was expressed as percentage of live cells of total number of counted spermatozoa; besides, sperm morphological anomalies were observed.

Table 1. Means ± SE of fowl sperm motility (%) and forward progressive motility (score 1-4) of extended samples by standard diluent or supplemented lycopene standard diluent during in vitro storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Motility FP</td>
<td>Motility FP</td>
<td>Motility FP</td>
</tr>
<tr>
<td>Lake</td>
<td>77.50±1.95</td>
<td>3.00±0.18</td>
<td>70.00±1.95</td>
</tr>
<tr>
<td>Lycopene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 µg/L</td>
<td>75.00±1.95</td>
<td>3.12±0.18</td>
<td>71.25±1.95</td>
</tr>
<tr>
<td>250 µg/L</td>
<td>77.50±1.95</td>
<td>3.25±0.18</td>
<td>71.25±1.95</td>
</tr>
<tr>
<td>500 µg/L</td>
<td>77.50±1.95</td>
<td>3.00±0.18</td>
<td>71.25±1.95</td>
</tr>
<tr>
<td>Fresh semen 1</td>
<td>77.50</td>
<td>3.00</td>
<td></td>
</tr>
</tbody>
</table>

1Time = 0 h.
The lycopene supplement did not significantly improve \( P > 0.05 \) sperm motility and forward progressive motility of stored semen. Number of replicate observations = 6.

Discussion

It is known that improvement of semen characteristics quality depends on antioxidant capacity to limit the damaging effects of lipid peroxidation (DONOGHUE and DONOGHUE, 1997). The antioxidant protective system of avian seminal plasma was studied in an in vitro model system (SURAI et al., 1998). Moreover, SURAI et al. (1998a, b) reported that water-soluble antioxidants as vitamin C, glutathione and enzymes are involved and that they play a central role against peroxidation damages. According to the present results, the supplementation of lycopene, as a water-soluble powder at tested concentrations did not improve motility and forward progressive motility parameters compared to a standard avian diluent, Lake. The effect of lycopene inclusion was interesting for viability parameter; at 500L concentration the viability after 24 h was higher than for the other aliquots. Probably, this confirms its protective effect against cell damage during storage.

In recent works, which aimed to improve the quality of stored avian semen, different water-soluble antioxidants supplemented to commercial diluents as vitamin C, green tea extract and catechin were object of investigations (DONOGHUE and DONOGHUE, 1997; MALDIAN et al., 1998). Vitamin C tested for a concentration ranging from 1 to 400 µg/mL at 5°C did not improve turkey semen parameters suggesting that the damage in vitro does not depend
on peroxyl radicals located in the aqueous phase, because vitamin C reacts against these radicals as a powerful antioxidant (Donoghue and Donoghue, 1997). The inclusion of green tea extract and catechin (100ppm) in an avian standard diluent (Lake, the same extender as in our research), reduced – compared to Lake without supplementation – the proportion of damaged cells after 48 h at room temperature in turkey semen samples, but it did not improve motility parameters (MalDjian et al., 1998). We found the same positive effect on spermatozoa survival with the lycopene 500L supplemented extender. This result is confirmed by our recent research (Mangiagalli, unpublished data), which compared different levels of lycopene supplement concentration (500µg/mL vs. 750µg/mL). Also, in this trial the 500L concentration shows an improvement in semen viability and kinetic characteristics even when compared to higher levels of lycopene supplementation (73.95±0.96 vs. 71.08±0.96; 57.5±2.08 vs. 54.00±2.08; 2.25±0.17 vs. 2.00±0.17). This can be considered as a further confirmation of the obtained results. In this trial, lycopene was used as an alternative molecule to α-tocopherol and ascorbic acid for its analogue properties as a radical-scavenging antioxidant. However, it is interesting to underline that there is a synergism among carotenoids, vitamin E and vitamin C supporting their mutual activities.

Table 2. Means ± SE of fowl sperm viability (%) of extended samples by standard diluent or supplemented lycopene standard diluent during in vitro storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of in vitro storage</th>
<th>1 h</th>
<th>6 h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake</td>
<td></td>
<td>82.70±0.95³x</td>
<td>76.12±0.95³y</td>
<td>71.83±0.95³z</td>
</tr>
<tr>
<td>Lycopene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100µg/L</td>
<td></td>
<td>78.69±0.95³xb</td>
<td>76.90±0.95³x</td>
<td>72.95±0.95³yh</td>
</tr>
<tr>
<td>250µg/L</td>
<td></td>
<td>82.95±0.95³xa</td>
<td>76.55±0.95³y</td>
<td>71.04±0.95³zb</td>
</tr>
<tr>
<td>500µg/L</td>
<td></td>
<td>83.16±0.95³xa</td>
<td>77.00±0.95³y</td>
<td>75.08±0.95³y</td>
</tr>
<tr>
<td>Fresh semen¹</td>
<td></td>
<td>84.56±2.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Time = 0 h.
³a,b Means in columns with different superscript differ significantly (P < 0.05).
³x,z Means in rows with different superscript differ significantly (P < 0.01).
Number of replicate observations = 6.

on peroxyl radicals located in the aqueous phase, because vitamin C reacts against these radicals as a powerful antioxidant (Donoghue and Donoghue, 1997). The inclusion of green tea extract and catechin (100ppm) in an avian standard diluent (Lake, the same extender as in our research), reduced – compared to Lake without supplementation – the proportion of damaged cells after 48 h at room temperature in turkey semen samples, but it did not improve motility parameters (MalDjian et al., 1998). We found the same positive effect on spermatozoa survival with the lycopene 500L supplemented extender. This result is confirmed by our recent research (Mangiagalli, unpublished data), which compared different levels of lycopene supplement concentration (500µg/mL vs. 750µg/mL). Also, in this trial the 500L concentration shows an improvement in semen viability and kinetic characteristics even when compared to higher levels of lycopene supplementation (73.95±0.96 vs. 71.08±0.96; 57.5±2.08 vs. 54.00±2.08; 2.25±0.17 vs. 2.00±0.17). This can be considered as a further confirmation of the obtained results. In this trial, lycopene was used as an alternative molecule to α-tocopherol and ascorbic acid for its analogue properties as a radical-scavenging antioxidant. However, it is interesting to underline that there is a synergism among carotenoids, vitamin E and vitamin C supporting their mutual activities (Sural, 2002). This is suggested by Long and Kramer, (2003), as well. These authors reported that supplemental vitamin E (10 and 40 µg/mL) in turkey semen extender yielded inconsistent results in stored sperm mobility, viability and lipid peroxidation. Considering the results of fertility, they supposed a negative influence of lipid peroxidation on sperm energetics affecting turkey sperm fertilizing ability and concluded that the addition of vitamin E alone was not sufficient to prevent lipid peroxidation.

By these reasons, we think that further investigations are needed in order to better understand the biochemical action of lycopene and its possible utilization to improve stored poultry semen characteristics.

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Summary

Chicken and turkey sperm contains high amounts of PUFAs and spontaneous peroxidation occurs during in vitro storage. For this reason, the addition of antioxidant to diluent takes special interest in the improvement of commercial extenders. The effects of lycopene added to fowl sperm was the object of this study. Semen of 13 roosters, from 36th to 43rd week of age, was collected and pooled. Fresh semen was evaluated at 0 h time and 4 aliquots of 500µL were diluted to 2x10⁹ sperm/mL by diluent Lake or Lake and lycopene at different concentrations (500µg/mL, 250µg/mL and 100µg/mL) and stored at 13±1°C in a water bath for 24 h. Motility, forward progressive motility and viability were evaluated at 1 h, 6 h and 24 h.

Sperm motility and forward progressive motility were not significantly (P > 0.05) affected by lycopene inclusion. For 500µg/mL treatment the viability parameter was significantly (P < 0.05) higher than for Lake or Lake with 250µg/mL and higher for Lake with 100µg/mL. Besides, among the treatments the 500µg/mL of lycopene reduced the viability loss between 6 h and 24 h of incubation. The results of the present study suggest positive effects of lycopene supplementation to extender on fowl sperm survival during liquid storage.

Key words

Chicken, sperm, diluent, lycopene, in vitro storage

Zusammenfassung

Einfluss von Lycopene auf die Eigenschaften von Geflügel sperma während der in vitro Lagerung

Hühner- und Putensperma enthält große Mengen an mehrfach ungesättigten Fettsäuren (PUFA), wodurch es zu antioxidativen Prozessen während der in vitro Lagerung


Stichworte

Huhn, Sperma, Verdünnung, Lycop, in vitro Lagerung

References


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