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EFFECT OF ANTIOXIDANTS ON SPERMATOLOGICAL PARAMETERS IN TWO DIFFERENT CARP SPECIES AFTER SHORT-TERM STORAGE

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Summary

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In this study, the effects of different antioxidants on the motility of grass and common carp semen were evaluated after short-term storage. Semen was collected from males by the abdominal massage. After determination of main semen characteristics, the pooled ejaculates were diluted with 350 mM glucose, 30 mM Tris-containing diluent. To the semen diluents, taurine (25, 50 and 75 mM), ascorbic acid (0.1, 0.3 and 0.5 mg/mL) and oxidised glutathione (0.5, 1 and 2.5 mM) were added. The refrigerated semen was stored for 72 hours at +4°C. Semen motility was recorded at 0, 24, 48 and 72 hours. These results indicate that that the addition of 2.5 mm oxidised glutathione for common carp (*Cyprinus carpio*) and 50 mm taurine for grass carp (*Ctenopharyngodon idella*) as antioxidants have more positive effects on spermatological parameters.

Key words: common carp, grass carp, short term storage, spermatological parameters

INTRODUCTION

Short-term preservation of fish sperm is generally useful from the commercial point of view and facilitates various hatchery operations. The short-term storage of sperm at low temperature (4ºC) is mostly applied in short distance transport of gametes collected in different locations, in synchronising the timing of obtaining good quality of gamete collection from males and females during artificial insemination, in avoiding the aging of sperm, in facilitating hatchery operations, also in experimental programs for genetic studies (Rana, 1995).

The important problem encountered in fish breeding is that the genetic structure cannot be maintained for a long period of time because neither a regular selection program nor a large number of fertilised eggs and juvenile fish are taken in order to profit from business owners.

Controlled fish production has reached considerable size in order to meet animal protein needs. The genetic potential of

fish, selection can be improved by crossing between appropriate individuals and manipulation of gametes. Some of these applications require preservations of the semen (Akcay *et al*., 1995).

This study was carried out to investigate the effect of 3 different antioxidant additions on 2 different carp species (*Cyprinus carpio* and *Ctenopharyngodon idella*) under short term storage conditions to spermatological parameters.

MATERIAL AND METHODS

During the breeding season, 15 male carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*), which reached sexual maturity were used. Spermatozoa were collected without anesthetic agent using abdominal massage and quantity (mL), motility (%), duration of life (s), density ($\times 10^9$/mL), viability, pH values were determined. Semen were diluted 1:10 (semen:diluent) with 350 mM glucose, 30 mM Tris-containing diluent. To the semen dilutions, 25, 50 and 75 mM TAURINE, ascorbic acid (0.1, 0.3 and 0.5 mg/mL) and oxidised glutathione (0.5, 1 and 2.5 mM) were added. Semen motility was recorded at 0, 24th, 48th and 72nd h. Ethics Committee Permit: The application was granted permission from the T.C. Mustafa Kemal University Animal Experiments Local Ethics Committee (11.12.2009; No 2009-6/66.)

Adult males of carp and grass carp aging from Adana State Fisheries Experimental Station, Adana, Turkey, were used (Fig. 1). In the pre-spawning season period the parenteral brood fish were kept separately in small ponds.

Semen was collected from hand-stripping method after a single injection of 2 mg/kg carp pituitary extract, CPE (Saad & Billard, 1987). Milt was collected into

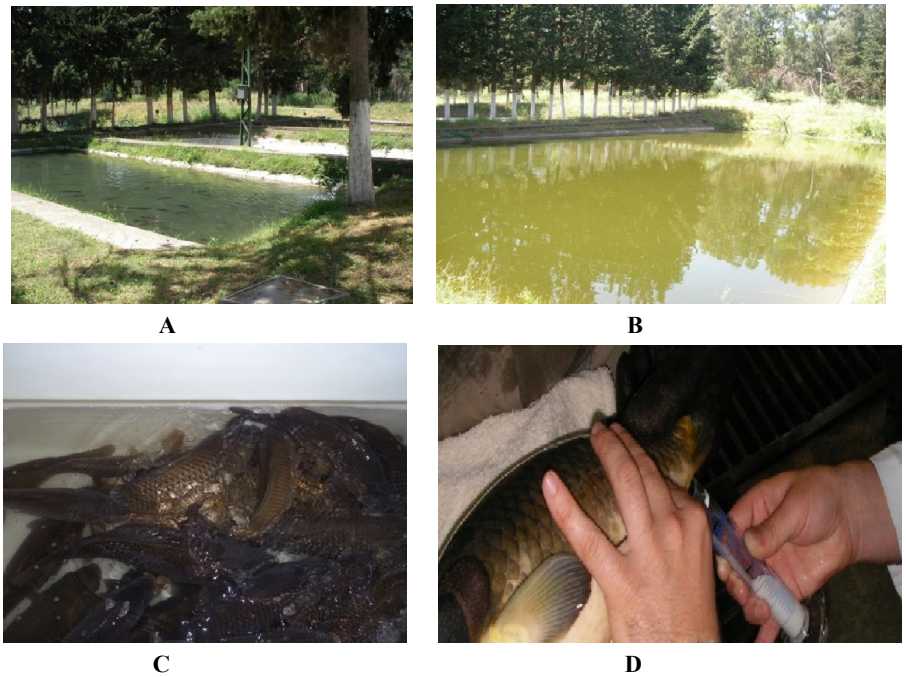

Fig. 1. A. Common carp pond; **B**. Grass carp pond; **C.** Common carp tank; **D.** Semen collection.

50 mL calibrated glass. Samples that were contaminated with faeces or urine were discarded.

Sperm motility was evaluated using a microscope (x400) and was expressed as a percentage of motile spermatozoa. An activating solution, 0.3% NaCl was used for estimating motility. Samples below 70% of motile spermatozoa were discarded. Movement duration was estimated using a sensitive chronometer (1/100 s). Motile spermatozoa and duration of movement were evaluated according to the following criteria: 1) Mass progressive motility when most of the spermatozoa were still actively swimming with progressive movement. 2) Total duration of motility until most spermatozoa stopped swimming.

The sperm concentration was estimated using the haemocytometric method and expressed as spermatozoa number $\times 10^9$/mL. pH was measured by indicator papers (Merck 5.5–9.0)

Data for percentage of sperm motility and fertilisation were transformed by angular transformation prior to statistical analysis by SPSS 10.0 software. Differences between parameters were analysed by ANOVA. Significant means were subjected to a multiple comparison test (Duncan) for post hoc comparison at a level ofa = 0.05. All analyses were carried out using SPSS 10 for Windows statistical software package.

RESULTS AND DISCUSSION

According to the results (Table 1 and 2), it was concluded for short term storage that the addition of 2.5 mM Oxidizing Glutathion to carp (*Cyprinus carpio*) and 50 mM Taurine for grass carp (*Ctenopharyngodon idella*) as antioxidants have more positive effects on spermatological parameters.

In living creatures carrying out respiration with oxygen, components and metabolites, called as reactive oxygen species (ROS), occur in cells. ROS detrimentally affect cell constituents; lipids, protein, carbohydrates and also DNA. In cells, the main sources of ROS are mitochondria, in which oxygen is used in order to produce ATP. Singlet oxygen (O^{-2}), the source of all ROS, comes out in mitochondria during respiration. Cells have developed antioxidant defence systems against singlet oxygen and ROS during evolution. In this way, detrimental effects of oxidative stress are reduced on cell base. Although the spermatozoon, whose cytoplasm are lost during spermatogenesis, is lacking antioxidant defense systems, spermatozoa have higher mitochondrial activity compared to somatic cells. Moreover, membrane of spermatozoa is rich from unsaturated fatty acids, vulnerable to oxidative stress. Furthermore, there is not any repairing mechanism in spermatozoa in order to obviate cell injuries caused by ROS. The main source of antioxidants is seminal plasma in semen and its protective effects is limited. It is exact that

Table 1. Spermatological properties of collected ejaculates (Mean ± SEM)

	Volume (mL)	Motile Cells (%)	Duration of Motility	Concentration ($\times 10^9$/mL)	pH
Common Carp	14.3±1.4	94.6±2.3	249.5±1.6	11.5±2.4	7.4±0.2
Grass Carp	12.2±1.1	92.7±1.4	325.1±3.1	14.9±1.7	7.4±0.1

Table 2. Effects of antioxidants on sperma motility (Mean ± SEM)

	Common carp semen motility (%)				Grass carp semen motility (%)			
	0 hour	24 hour	48 hour	72 hour	0 hour	24 hour	48 hour	72 hour
Taurine 25 mM	82.5± 5.2[a]	61.2± 2.1[a]	42.4± 1.3[a]	19.2± 2.3[a]	82.5± 4.1[a]	62.3± 2.1[a]	44.4± 1.4[a]	19.1± 2.1[a]
Taurine 50 mM	82.4± 4.3[a]	59.4± 4.2[a]	39.1± 2.1[a]	16.1± 2.2[a]	92.5± 5.5[a]	73.5± 4.1[a]	53.6± 3.5[a]	27.3± 2.2[a]
Taurine 75 mM	87.4± 2.1[a]	49.4± 2.3[b]	22.1± 2.1[b]	0[b]	81.3± 3.1[b]	42.4± 2.3[b]	21.1± 1.2[b]	0[b]
Ascorbic acid (0.1 mg/mL)	83.5± 3.4[a]	47.4± 2.4[c]	19.3± 2.1[b]	3.4± 0.1[b]	83.5± 3.4[b]	41.4± 2.4[b]	19.1± 2.1[b]	8.1± 1.1[c]
Ascorbic acid (0.3 mg/mL)	87.5± 3.5[a]	46.4± 1.3[c]	24.3± 1.6[b]	0[b]	81.5± 2.5[b]	42.4± 1.3[b]	20.1± 1.8[b]	0[b]
Ascorbic acid (0.5 mg/mL)	83.3± 2.9[a]	46.2± 4.3[c]	23.2± 1.4[b]	0[b]	83.3± 2.6[b]	43.4± 1.3[b]	23.2± 2.4[b]	0[b]
Oxidised glutathione (0.5mM)	82.5± 3.4[a]	50.1± 2.9[c]	19.1± 1.4[b]	4.3±1.2[b]	82.5± 2.4[b]	54.4±2.3[c]	19.1± 1.1[b]	11.3± 1.2[c]
Oxidised glutathione (1mM)	87.4± 3.1[a]	51.4± 4.3[c]	27.5± 2.4[b]	15.4± 1.1[a]	83.4± 3.1[b]	53.4±2.3[c]	25.5± 2.4[b]	10.4± 1.0[c]
Oxidised glutathione (2.5mM/)	91.4± 6.4[b]	72.3± 3.1[d]	54.4± 1.4[c]	29.1± 2.1[c]	82.4± 2.9[b]	52.3±2.1[c]	33.4± 1.3[c]	11.1± 2.1[c]

spermatozoa are exposed to oxidative stress during short or long term storage. Antioxidative precautions against ROS, with knowledge of oxidative stress and its detrimental effects on spermatozoa, may improve success of short and long term storage of sperm (Ari & Ozturkler, 2015)

During preservation, semen is exposed to cold shock and atmospheric oxygen, which in turn increases susceptibility to lipid peroxidation that is due to increased production of ROS. Excessive production of ROS during cryopreservation has been associated with reduced post-thaw motility, viability, membrane integrity, fertility, and antioxidant status Antioxidants play an important role in the protection of sperm and can neutralize the effects caused by the cryopreservation procedure. (Michael *et al.,* 2007).

However, there is a lack of information regarding the effect of commonly used antioxidants on spermatological parameters and antioxidant activity in fish. (Yavas *et al.,* 2014).

In the present study, the addition of 2.5 mM oxidised glutathione for short carp (*Cyprinus carpio*) and 50 mM Taurine for grass carp (*Ctenopharyngodon idella*) as antioxidants have more positive effects on spermatological parameters. Similar effects were reported in European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) semen (Cabriata *et al.,* 2011; Paramo *et al.*, 2012).

Supplementation of extenders with various antioxidants at different ratios improved fertilising capability of cryopreserved semen significantly. This study indicated that antioxidants and their concentrations are very important in carp semen preservation. Our results suggest a species-specific effect probably depending not only on the type of antioxidant added but also on its concentration. More re-

search is needed to understand the precise physiological role of these antioxidants and others in sperm protection.

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