Hatchability of pheasant eggs fertilized with cryopreserved semen from dietary manipulated males

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It is well known that avian spermatozoa are sensitive to lipid peroxidation due to the kind of phospholipids that form their membrane. Consequently protection against peroxidation, especially during the freezing-thawing procedure is necessary to maintain the structural integrity of the spermatozoa.

The aim of the present investigation was to test *in vivo* the fertilizing capacity of cryopreserved semen obtained from males fed on two diets differing for selenium/vitamin E ratio (Se/Vit E). A basal commercial feed (11.51 MJ/Kg of M.E., 19% of C.P.) with 1% fish oil, was enriched with 40 mg vitamin E plus 0.1 mg selenomethionine per Kg of feed or with 200 mg vitamin E plus 0.3 mg selenomethionine per Kg of feed, thus to obtain 0.0025 and 0.0015 Se/Vit E, respectively. The pheasants were fed the diets for one month and then semen was collected from the males and processed for the freezing procedure in pellets as described by Castillo *et al*. (2011). 100µl semen pellets were stored in liquid nitrogen for one year and then thawed by a hotplate at 75 °C and used for artificial inseminations (AIs). The thawing procedure was performed as described by Marzoni *et al*. (2009).

Hatchability was evaluated on eggs laid by 10 female pheasants in a 19-day period. The females were divided into two groups according to the dietary semen group received. Each female received doses of 31 to $37x10^6$ live normal thawed spermatozoa. The first two AIs were performed on successive days and thereafter AI was carried out twice a week, for a total of 6 AIs. Sperm qualitative parameters were assessed as described by Marzoni *et al*. (2009). Data were subjected to analysis of variance, percentage data were arcsine transformed prior to analysis. Egg parameters expressed in percentages were compared by the chi^2 test.

Table 1 reports some qualitative parameters of fresh and thawed semen in relation to the dietary treatment. In fresh semen there were no significant differences for any of the parameters as previously observed by Marzoni *et al.* (2010). In the thawed semen, less dead, more normal and better performing cells were obtained in the 0.0015 Se/Vit E group. On the contrary, results of the *in vivo* study (Table 2), showed no significant differences between the dietary semen groups. As egg production was the same between the two female groups assigned to test the cryopreserved semen, the environment within the oviduct of the female allowed the expression of some unknown male cell defects, such as for example a defective chromatin in sperm with microscopically normal morphology.

In conclusion, in this study more resistant and better performing cell *in vitro* was found in cryopreserved semen from males fed the diet supplemented with 200 mg Vitamin E plus 0.3 mg organic Selenium per kg of feed.

References

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Table 1 Characteristics of fresh and thawed semen and the effect on semen characteristics of two different diets offered to two groups of male pheasants at one month before the ejaculates collection

		Dietary Se/Vit E	
		0.0025 mean ± SD	0.0015 mean ± SD
Fresh semen			
pH		8.30 ± 0.17	8.30 ± 0.04
Sperm concentration	10^{*9}/mL	7.30 ± 0.69	8.45 ± 0.32
Dead spermatozoa	(%)	18.92 ± 4.25	16.15 ± 3.22
Morphologically normal spermatozoa	(%)	61.55 ± 7.28	58.73 ± 4.42
Mobility	(A_{550nm})	0.298 ± 0.045	0.330 ± 0.034
Thawed semen			
Dead spermatozoa	(%)	71.71^{A} ± 3.25	65.80^{B} ± 3.15
Morphologically normal spermatozoa	(%)	10.90^{B} ± 1.50	18.67^{A} ± 0.98
Mobility	(A_{550nm})	0.042^{B} ± 0.005	0.066^{A} ± 0.002

A,B: *P<0.01*

Table 2 Fertility and hatchability of pheasant eggs fertilized by dietary manipulated cryopreserved semen

	Dietary Se/Vit E	
	0.0025	0.0015
set eggs n.	81	85
	(%)	
fertility	33.33	$25.88_{n.s}$
hatchability on fertile eggs	29.63	$31.82_{n.s}$

ns: not significant