American-Eurasian J. Agric. & Environ. Sci., 2 (1): 48-50, 2007
ISSN 1818-6769

Cytochrome C Affects The Viability and Fertility of Bull Semen

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Abstract: This study was carried out to determine the effect of antioxidant cytochrome C, by supplement at concentration 1.5 mg ml^{-1} to Nagase-Niwa's diluent on motility, viability and fertility of bull spermatozoa. Cytochrome C improved significantly viability of spermatozoa of fresh semen and after deep freezing. Cytochrome C does not influence significantly on the viability of spermatozoa during deep freezing. The conception rate of cows after calving is increased with 8.3% and with 5.4% higher at non return ($p<0.05$).

Key words: Cytochrome C · spermatozoa · deep freezing · viability · fertility

INTRODUCTION

The investigations of number of authors revealed that the activity of enzymes systems is directly related to viability and fertilizing capacity of spermatozoa. The capacity of spermatozoa to perform independent movement is a characteristic physiological feature related to their biological function-to fertilize the ovule. This fact justifies the interest shown by many authors who attach great significance to the enzymes taking part in the processes of fertilization [1, 2].

An important reason for the decrease in the fertility during storage of semen is formation of peroxides in presence of oxygene radicals [3]. The deep freezing increases sensitivity to lipid peroxidation [4, 5].

A large number of antioxidants have been tested in an attempt to minimize the peroxidation [6]. Superoxide dismutase (SOD) and glutathione peroxidase-glutathione reductase-system which plays a protective role against damage-associated with oxygen metabolism [4]. Cytochrome C could also function as a potent antioxidant whereas is a role beyond its utility as an electron carrier [7]. Under normal physiological conditions, cytochrome C is attached to the outside of the inner mitochondrial membrane to function as an electron carrier-with a rise of Reactive Oxygene Species (ROS) levels [8]. With a rise of mitochondrial ROS levels, cytochrome C detaches from the inner mitochondrial membrane and is capable of oxidizing O_2 to form molecular oxygene [9].

The antioxidants-SOD, cytochrome C, catalase, glutathione peroxidase increase both the survival and acrosome integrity of spermatozoa during storage at 5 and 25°C [6]. Therefore it is important to determine whether the improved viability of spermatozoa would be reflected on the fertility. In the present study we examined benefits of cytochrome C-water soluble antioxidant for the viability and the fertility of bovine spermatozoa after the deep freezing.

MATERIALS AND METHODS

Collection, dilution and deep freezing of semen: Bull semen from 2 Holstein Frisian bulls was used for Artificial Insemination (AI). The semen was collected by artificial vagina and its semen quality was assessed and accepted as a donator, every ejaculate had full quality: volume >5.0 ml, macroscopic good visual mass, activity, sperm concentration > 0.8×10^9 and progressive sperm motility >70%. For the freezing, fresh bull semen was diluted in Nagase-Niwa's diluent 1:3 (semen:diluent) at 30°C. After the equilibration for 4 h at 3°C the semen was frozen according to the technology of Nagase-Niwa.

Thawing of deep frozen semen: The semen was thawed at 39°C as follows: sodium citrate -2.8 g, dest. water -100 ml. The diluent was divided into two equal parts: without and with 1.5 mg ml^{-1} cytochrome C (Fluka, Switzerland). The pH-values of medium were 7.5 and osmolarity 201 m Osm kg mol 1^{-10} (Cryoscopic osmometer of medium Osmomat-30, Gonotec, Berlin, Germany). The frozen semen (cytochrome C is supplemented in the medium during the freezing) in pellet at 0.15 ml, containing $10\text{-}15\times10^6$

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Table 1: Effect of cytochrome C on the viability and fertility of bull spermatozoa (Experiment 1)

	Motility of the spermatozoa (%)			Viability of the spermatozoa at 39°C (min)				Fertility of the spermatozoa		
								Without CHC	With 1.5 mg ml^{-1} CHC	
	Without	With 1.5 mg ml^{-1}	Distinc-	Without	With 1.5 mg ml^{-1}	Distinction		Cows calved/total	Cows calved/total	
Variants	CHC	CHC	tion	CHC	CHC	min	%	insemination	insemination	Distinction
Variant A	76.0±8.1	76.0±8.1	-	491±12.92**	654±22.40**	+163	33.2	-	-	-
Variant B	35.5±0.8	37.1±1.2	+1.6	311±12.56	357±23.10	+46	14.8	-	-	-
Variant C	35.5±0.8*	41.0±1.8*	+5.5	311±12.56**	483±27.40**	+172	55.3	64/144(44.4%)	41/77(53.2%)	+8.3%

* p<0.01 ** p<0.001, Variant A - Fresh semen A[1] - (Cytochrome C is supplemented to the fresh semen), Variant B - Frozen semen B [1] (Cytochrome C is supplemented to the medium during the freezing), Variant C - After the deep freezing of the semen C[1] - (CytochromeC is supplemented to the medium for the thawing), Abbreviation-CHC - Cytochrome C

Table 2: Effect of cytochrome C on the viability and fertility of bull spermatozoa after the deep freezing (Experiment 2)

	Variants				
			Distinction		
Indicators	Without CHC	With 1.5 mg ml^{-1} CHC	min	%	p
Motility of the spermatozoa (%)	36.0±1.2	42.0±1.5	-	+6	= 0.050
Viability of the spermatozoa at 39°C (min)	352±2.8	516±3.1	+164	+46.6	= 0.001
Fertility of the spermatozoa (NR)	723[a]/1203[b]	738/1127 (65.5%)	-	+5.4	= 0.050

[a] - cows in pregnancy, [b] - artificial inseminated cows, Abbreviation: CHC - Cytochrome C

progressive motile sperm was thawed at 39°C in 0.5 ml of the diluent. For the investigations of the viability of thawed semen, the pellet at 0.15 ml was placed in 0.5 ml medium without and with cytochrome C (cytochrome C is supplemented in the thawing medium). All aliquots were incubated at 39°C and spermatozoa were assessed for motility.

Fertility trials: The artificial insemination of cows (Holstein Frisian) was performed by bimanual method. Estrous onset was detected and noted by an experienced person, who carried out continuous observations through watching behavior and clinical and gynecological symptoms characteristic for the estrous in females of this species [10]. The cows were inseminated with deep frozen semen in the pellet from 0.15 ml in volume with 10-15×10^6 progressively motile spermatozoa. The semen was thawed at 39°C in 0.5 ml of diluent without cytochrome C (control sample) and in the sample containing 1.5 mg ml^{-1} cytochrome C (cytochrome C is supplemented in the thawing medium). The fertility rate was determined at first artificial insemination after calving (experiment 1) and at Non Return (NR) 60 days after insemination (experiment 2).

RESULTS AND DISCUSSION

Experiment 1: Data analysis showed that cytochrome C significantly influenced on the viability of spermatozoa during incubation at 39°C in fresh semen and after deep freezing. Cytochrome C does not influence significantly on the viability of the spermatozoa during deep freezing (Table 1). As it is seen from Table 1 in fresh semen and after deep freezing there are considerable differences in the viability of spermatozoa. In fresh semen in medium containing cytochrome C -1.5 mg ml^{-1} the viability of the spermatozoa is higher with 33.2%, compared to the control samples (p<0.001) and in the semen after deep freezing is respectively with 55.3% (p<0.001).

The conception rate of cows after insemination without and with 1.5 mg ml^{-1} is presented at Table 1. The fertility is increased with 8.3%.

Experiment 2: In the medium containing cytochrome C, the motility of spermatozoa after deep freezing is with 6% higher (p<0.05) and the viability is higher with 46.6% (p<0.001), compared to the control samples (Table 2).

The conception rate of cows NR after insemination without and with 1.5 mg ml^{-1} is presented at Table 2.

In experiment 2, the fertility is increased with 5.4% ($p<0.05$).

A large number of antioxidants have been tested in an attempt to minimize peroxidation [3, 6, 11, 12]. In the present study we examined the benefits cytochrome C for the fresh bull semen and after deep freezing. There is no significant effect of C when it is at supplement during deep freezing of the spermatozoa. It was important to determine whether the improved viability of spermatozoa would be reflected on the fertility. The ability of cytochrome C to improve the fertilizing capacity of spermatozoa *in vivo* was examined in experiment 1 and 2.The fertilization rates were better at the insemination with semen extended with diluent containing cytochrome C, than in its absence after deep freezing. The reduction in fertilizing capacity of spermatozoa after deep freezing may reflect either changes in the nature of the motility the cells, or the changes in their membranes. The main beneficial effects of cytochrome C may be associated with sperm ageing [13]. In addition cytochrome C function as a potent antioxidant that scavenges ROS in the mitochondria. The presence of high levels of cytoplasmic GSH (gluthatione) maintain Cytochrome C in an inactive and it has been suggested that cytochrome C will only induced programmed cell death if it is present in the cytoplasm in the oxidized state [14]. Indeed, the redox status of cytochrome C and thus its structure can be altered by the presence of ROS and reduced gluthatione [15]. Cytochrome C improved the survival of spermatozoa in liquid storage at both 5 and 25°C [6].

From the results of present study we can recommend the supplement of different antioxidants to the diluents at storage and after the deep freezing of the sperm for improving the quality of the semen used in artificial insemination.

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