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Short communication

Relation between nitric oxide (NO) level in semen and certain properties of boar spermatozoa stored at 17°C

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Abstract

The aim of this study was to measure the NO level in boar semen held in a liquid state and to determine its putative relation to spermatozoa motility, plasma membrane integrity, mitochondrial membrane potential and ATP content. Generally, the percentage of spermatozoa which generated nitric oxide gradually increased, while NO level in the surrounding medium declined during the liquid preservation. NO generation in semen preserved in BTS was higher as compared to those in Androhep®Plus. We demonstrated the positive correlation between the NO level in fresh spermatozoa and their quality. We also showed negative correlation between nitric oxide level in spermatozoa preserved in BTS and sperm cells motility as well as plasma membrane integrity. Results obtained in this study confirm that NO may affect sperm physiology in a dualistic manner.

Key words: nitric oxide, liquid storage, boar spermatozoa quality

Introduction

Metabolic activity of sperm cells during storage at 18°C result in the accumulation of metabolic end-products including reactive oxygen species (ROS) in the semen. Reactive nitrogen species (e.g. nitric oxide, nitrogen dioxide, peroxynitrite, peroxynitrous acid) are free nitrogen radicals which were classified as a subclass of ROS class (Sikka 2001). Nitric oxide (NO) is synthesized from endogenous L-arginine by nitric oxide synthase (NOs) (Mehraban et al. 2005). Three distinct isoforms of NOS (nNOS - neuronal, iNOS - inducible and eNOS - endothelial) have been purified, sequenced, and partially characterized. They are found in the Sertoli cells, germ cells in the seminiferous epithelium, Leydig cells, myofibroblasts, myoid cells, endothelial cells and spermatozoa. Presence of eNOS, iNOS, and nNOS in the testis is an indicator of the importance of NOS for spermatogenesis (Doshi et al. 2012). At physiologic levels NO is important in sperm capacitation. It may also have an anti-apoptotic effect in sperm (Wang et al. 2014). On the other hand, the majority of findings demonstrate that elevated amounts of nitric oxide decrease motility and are associated with increased sperm toxicity and apoptosis (Mehraban et al. 2005). Thus, the aim of this study was to assess changes of nitric oxide content during liquid storage of boar semen.

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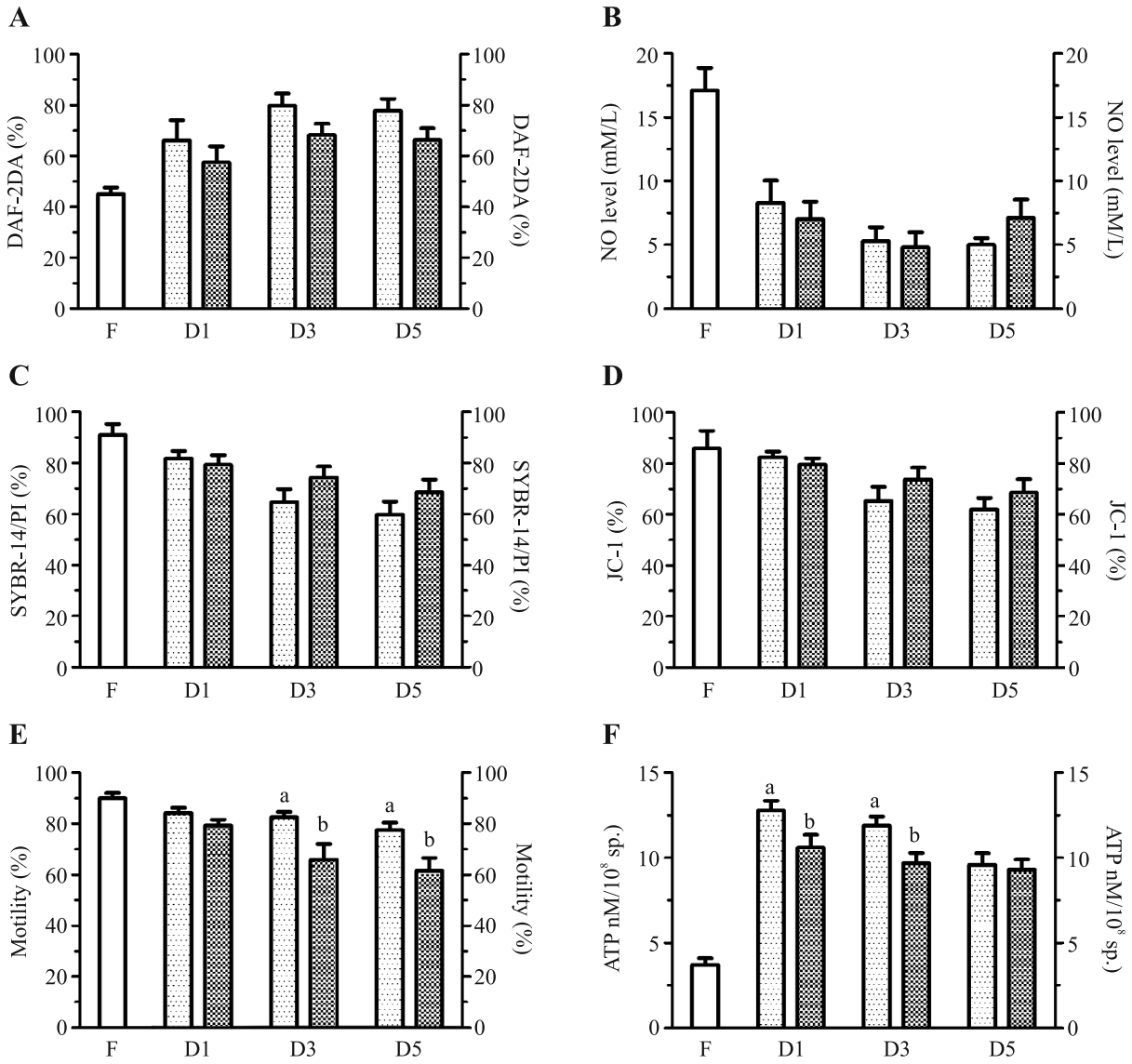

Fig. 1. Comparison of certain biological and biochemical properties of semen kept in a liquid state in BTS (▒) and Androhep®Plus (▒)
for five days; values (a, b) with different letters are statistically significant at p≤0.05.

Materials and Methods

Ejaculates were collected once a month by the gloved-hand technique. Experimental material were 9 sperm-rich fractions of 3 sexually mature boars (wbp race). After assessing spermatozoa concentration and motility one part of every ejaculate was extended with BTS (Minitube, Germany), whereas the other with Androhep®Plus extender (Minitube, Germany) to 30 × 10^6 spermatozoa/ml. Ejaculates were kept in a liquid state in 17°C in thermobox (WAECO, Minitube Gmbh, Germany) for five days. In fresh semen and at first, third and fifth day of storage semen samples were subjected to certain analyses. Spermatozoa motility was measured using computer-assisted sperm analysis (CASA) system (Hamilton-Thorne Research, IVOS version 12.3, USA). The percentage of membrane-intact spermatozoa was assessed with fluorescent dyes SYBR-14 and PI (Live/Dead Sperm Viability Kit, Molecular Probes), according to the method of Garner and Johnson (1995) with some modifications (Fraser et al. 2002). Spermatozoa membrane mitochondria potential was assessed with use of two fluorescent dyes JC-1 (Molecular Probes, USA) and PI, according to the method of Thomas et al. (1998). Spermatozoa were examined under a fluorescence microscope (Olympus CH 30, Japan). ATP content was determined with use of ATP Bioluminescence Assay Kit CLSII (Roche Molecular Biochemical Company, Germany) and a Junior bioluminometer (Berthold Technologies, Germany). Assessing of NO production by spermatozoa was conducted with DAF-2DA fluorescent dye in ac-

Table 1. Correlation coefficients between NO generation in fresh semen and certain properties of spermatozoa (values in bold type are statistically significant).

Parameters	DAF-2DA (%)	NO level (μM/L)
SYBR-14/PI (%)	0.06695	0.03847
JC-1/PI (%)	**0.67783**	0.00000
Motility (%)	**0.76070**	0.01339
ATP (nM/10^8sp.)	**-0.67783**	0.31629

Table 2. Correlation coefficients between NO generation in semen preserved in BTS (A) and Androhep®Plus (B) and certain properties of spermatozoa (values in bold type are statistically significant).

A

Parameters	D1		D3		D5	
	DAF-2DA (%)	NO level (μM/L)	DAF-2DA (%)	NO level (μM/L)	DAF-2DA (%)	NO level (μM/L)
SYBR-14/PI (%)	-0.48333	-0.00855	**-0.67783**	**0.79499**	-0.18333	0.20255
JC-1/PI (%)	-0.31667	-0.16245	-0.56667	**0.84274**	-0.51667	0.27850
Motility (%)	-0.29924	-0.08333	**-0.86083**	0.63364	**-0.68619**	0.28815
ATP (nM/10^8sp.)	-0.18647	-0.33914	-0.51046	0.05984	-0.34455	0.00851

B

Parameters	D1		D3		D5	
	DAF-2DA (%)	NO level (μM/L)	DAF-2DA (%)	NO level (μM/L)	DAF-2DA (%)	NO level (μM/L)
SYBR-14/PI (%)	0.13333	-0.16736	-0.09205	-0.60338	0.50000	-0.29538
JC-1/PI (%)	0.51667	-0.23431	0.41667	-0.28572	0.20000	-0.04219
Motility (%)	0.12552	**-0.89916**	0.457693	-0.12821	-0.03333	-0.43041
ATP (nM/10^8sp.)	-0.48333	-0.56067	-0.15063	0.38819	-0.31667	0.25318

cordance to the method of Lampiao et al. (2006). Stained spermatozoa were analyzed on InCell Analyser 2000 (GE Healthcare, Great Britain). NO content in plasma and extending medium was measured using reagents of Griess assay. Statistical analysis was performed with the package of Statistica programme (version 13.1, StatSoft Incorporation, USA). The data were analysed by ANOVA, followed by the NIR-Fischer test. Results were demonstrated as means and standard errors of the means (SEM). Correlation coefficients were assessed using Spearman's correlation module.

Results and Discussion

Results obtained in this study indicate that percentage of spermatozoa generating NO gradually increased, while NO level in the surrounding medium declined during the boar semen liquid preservation (Fig. 1 A, B). NO generation in semen preserved in BTS was higher as compared to those in Androhep®Plus (Fig. 1 A, B). The percentage of spermatozoa with intact plasma membranes and active mitochondria generally decreased as well as their motility and ATP content in case of both used extenders (Fig. 1 C, D, E, F). There were ascertained significant positive correlations ($p \leq 0,05$) between NO content in fresh semen and spermatozoa motility and membrane mitochondria potential (Table 1). From the third day of semen preservation in BTS, we showed negative correlations between NO content and the number of intact and motile sperm cells, while positive correlation between NO level in medium and mentioned features (Table 2 A). In case of Androhep®Plus we demonstrated negative correlation between NO level in medium and number of motile spermatozoa at first day of semen preservation (Table 2 B).

Although in physiologic conditions NO seems to act as a protectant against ROS-mediated damages, in situations of inappropriate NOS regulation, NO may exacerbate ROS-mediated pathology (Mehraban et al. 2005). It seems as if nitric oxide affects the sperm depending on both its concentration and the duration of its exposure to spermatozoa (Wang et al. 2014). Results obtained in this study suggest that endogenous NO formation in sperm cells preserved in short-term extender negatively influence both their motility and integrity from the third day of semen storage. It may be associated with the lack of BSA and HEPES in BTS composition, which reduce storage-dependent aging processes in Androhep®Plus. We also demonstrated relation between the level of NO and content of ATP in sperm cells preserved in BTS. Weinberg et al. (1995) found that NO decrease sperm motility in vitro, possibly by a mechanism involving the inhibition of cellular respiration, resulting in the depletion of sperm ATP. On the other hand some amount of endogenous NO is inevitable in spermatozoa physiology as demonstrated in fresh semen. Higher concentrations of NO in seminal plasma in infertile men are more likely to result in the inhibition of capacitation and they are also linked to a decrease in sperm metabolism (Doshi et al. 2012). In this study NO quantity in seminal plasma did not affect spermatozoa properties, however its level in the extending medium seemed to negatively affect sperm cells preserved in Androhep®Plus.

To sum up, NO is generated during liquid preservation of boar semen. Its excessive forming seems to influence certain parameters of the semen. Studies must be continued and extended with greater number of ejaculates.

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