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Short communication

Effects of the platelet-activating factor (PAF) supplementation on ATP content of cryopreserved bull spermatozoa (AI)

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Abstract

The aim of this study was to investigate the effect of PAF supplementation in semen extender on ATP content in cryopreserved bull spermatozoa used for artificial insemination at different time intervals. Cryopreserved semen was treated with different concentrations of PAF: $1x10^{-5}$M, $1x10^{-6}$M, $1x10^{-7}$M, $1x10^{-8}$M and $1x10^{-9}$M at 37°C. In the present work we showed that content of ATP in cryopreserved semen supplemented with $1x10^{-9}$M PAF was statistically significantly higher at 90 and 120 minutes of incubation in comparison to the control group ($p \leq 0.05$). Present study indicates the potential influence of PAF on ATP content in male spermatozoa via it's protective role towards mitochondria metabolic activity.

Key words: bull, spermatozoa, cryopreservation, PAF, ATP content

Introduction

The artificial insemination (AI) with cryopreserved semen is considered as the technology most commonly used in the controlled reproduction of cattle. The development of methods that ensured cell viability after storage for long periods leads to important progresses in studies involving different exogenous factors (Sugulle et al. 2006).

The platelet-activating factor (1-0-alkyl-2-acetyl-sn-glycerol-3-phosphoryl-choline) (PAF) is a unique signaling phospholipid that in addition to platelet activation, has been implicated in a number of biological processes (Kordan et al. 2003). Many studies indicate that PAF plays a key role in various reproductive processes such as ovulation, fertilization, embryo development, implantation and parturition (Bosch et al. 2009). Although the exact mechanisms are not clear, it has been demonstrated in laboratory animals and several domestic species that PAF can influence sperm function by affecting the motility, capacitation, acrosome reaction and fertility of spermatozoa (Roudebush and Diehl 2001, Kordan et al. 2010).

Present work is a continuation of our previous study, which concerned the effect of PAF on selected quality parameters of cryopreserved bull semen (Lecewicz et al. 2016).

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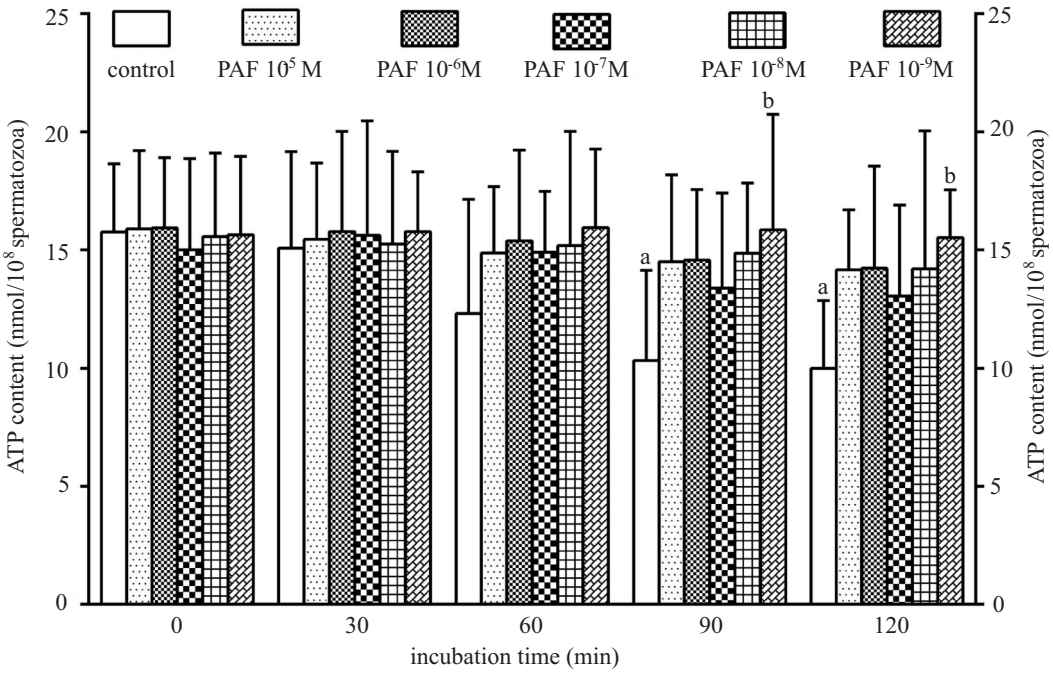

Fig. 1. ATP content of bull spermatozoa following treatment of cryopreserved semen with different concentrations of PAF within incubation time, values (a, b) with different letters are significant at p≤0.05.

The aim of this study was to investigate the effect of PAF supplementation in semen extender on ATP content in cryopreserved bull spermatozoa used for artificial insemination at different time intervals.

Materials and Methods

The experimental material was cryopreserved semen obtained from 10 bulls reared at the Animal Breeding and Insemination Centre in Bydgoszcz, used for insemination practice. Cryopreserved spermatozoa ($20x10^6$ spermatozoa/cm^3) were treated with different concentrations of PAF: $1x10^{-5}$M, $1x10^{-6}$M, $1x10^{-7}$M, $1x10^{-8}$M and $1x10^{-9}$M at 37°C. Cryopreserved semen without PAF supplementation was used as a control. ATP content in semen was examined at different time intervals: 0, 30, 60, 90 and 120 minutes. ATP content was determined using the Bioluminescence Assay Kit CLSII (Roche Molecular Biochemical) in accordance with the manufacturer's instructions. Values were expressed as the mean ± standard deviation (SD). The data were analysed by ANOVA, followed by the Duncan multiple comparison test using the Statistica software package (StatSoft Incorporation, Tulsa OK., USA). Differences between means were considered significant at p≤0.05.

Results and Discussion

The effect of different concentrations of PAF on ATP content in cryopreserved bull spermatozoa is shown in Fig. 1. Treatment of cryopreserved semen with $1x10^{-9}$M PAF showed consistently higher (p≤0.05) ATP content compared to the control. Statistically significant differences were observed at 90 and 120 minutes of incubation.

Thus far, sperm motility is the basic parameter of semen quality analyzed at insemination centres. It has been confirmed that sperm motility is dependent on intracellular ATP content, which appropriate levels are maintained by glycolysis in the cytoplasm of the principal sperm flagellum or through oxidative phosphorylation in the mitochondria of spermatozoa (Ford 2006). Furthermore, immunofluorescence study demonstrated the presence of PAF receptors, mainly in mid-piece and proximal head of ejaculated spermatozoa (Roudesbush and Diehl 2001).

In the present work we showed that content of ATP in cryopreserved semen supplemented with $1x10^{-9}$M PAF was statistically significant higher at 90 and 120 minutes of incubation in comparison to the control group. These findings are in accordance with our previous study, in which cryopreserved semen of bulls supplemented with mentioned PAF concentration was characterized by maintained spermatozoa

motility on appropriate high level (Lecewicz et al. 2016). These results are in line with Odeh et al. (2003), who demonstrated that treatment of stallion sperm with PAF at concentrations from 10^{-10} to 10^{-13}M, resulted in the best motility maintained after 120 min of incubation. Similar findings on spermatozoa motility were observed in cryopreserved boar sperm. Kordan et al. (2009) reported that spermatozoa exposured to 10^{-5}-10^{-6}M PAF concentration showed a significant increase in the sperm motility parameters. Furthermore, Kordan and Strzeżek (2006) observed an improvement boar sperm quality parameters during storage either at 5ºC and 16ºC after extender supplementation with PAF. Furthermore, in human and mouse it has been shown that spermatozoa responded with enhanced motility to their incubation with phospholipid, particularly sperm samples with poor initial motility.

In summary, as the midpiece of the spermatozoa is structure rich in mitochondria and is involved in maintaining sperm motility, it may indicate the potential influence of PAF on ATP content in male germinal cells via it's protective role towards mitochondria metabolic activity.

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