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Short communication

Effect of egg yolk and seminal plasma heparin binding protein interaction on the freezability of buffalo cauda epididymal spermatozoa

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Abstract

Egg yolk is routinely used in most of the extenders for cryopreservation of semen, but mechanisms of protection of spermatozoa by egg yolk are not very clear. Investigations with buffalo cauda epididymal sperm have shown that seminal plasma heparin binding proteins have detrimental effects during semen cryopreservation. The present study was conducted to investigate the effect of egg yolk on the detrimental effects of heparin binding proteins during cryopreservation of buffalo cauda epididymal spermatozoa. The results indicated that egg yolk was able to reduce the heparin binding proteins mediated cryoinjury in spermatozoa. One of the mechanisms of protection of spermatozoa from cryoinjury by egg yolk may be due to the inhibition of deleterious actions of heparin binding proteins on the spermatozoa.

Keywords: Buffalo; Cauda epididymal spermatozoa; Egg yolk; Heparin binding proteins; Seminal plasma; Cryopreservation

1. Introduction

Hen's egg yolk (EY) is an integral part of the extenders used for bovine semen. The low-density lipoprotein fraction (LDF) of EY appears to be responsible for protection of sperm against freezing damages (Moussa et al., 2002). Many of the hypotheses put forward for mechanisms of sperm protection by LDF suggest an association of LDF with sperm membranes (Foulkes, 1977; Cookson et al., 1984) while others suggest that EY-LDF prevents the loss of membrane phospholipids (Parks

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and Graham, 1992; Bergeron et al., 2004). Though the precise role of most components of semen extenders are known, the mechanism of protection provided by EY seems to be much complex and desires further studies.

Heparin binding protiens (HBP) are a group of proteins present in the seminal plasma, which are abundant on the surface of ejaculated spermatozoa but are rare on the plasma membrane of epididymal spermatozoa (Miller et al., 1990). HBP were found to modulate capacitation and zona binding ability of buffalo epididymal spermatozoa (Arangasamy et al., 2005). Manjunath and Sairam (1987) described three acidic proteins, which were secretory products of seminal vesicles and constituted the major proteins in bovine seminal plasma, collectively called as BSP proteins. Later it was found that they constitute the bulk of heparin binding proteins of seminal plasma (Chandonnet et al., 1990). The binding of BSP proteins to sperm membrane increases the number of heparin binding sites on the sperm surface. BSP proteins stimulate a dose and time-dependent (Manjunath and Therien, 2002) sperm membrane phospholipid and cholesterol efflux and prime them to undergo capacitation after further interaction with either heparin like glycosaminoglycans or high-density lipoproteins in the oviduct (Therein et al., 1999). Cholesterol is recognized to have a stabilizing effect on sperm membranes (Yeagle, 1985); hence its efflux would be expected to provoke reorganization or destabilization of the membrane (Manjunath and Therien, 2002). Experiments with HBP have revealed their deleterious effects on the freezability of epididymal spermatozoa (Harshan et al., 2006).

Bergeron et al. (2004) reported that BSP proteins bind to LDF of hen's EY, which decreases the binding of BSP proteins to sperm and prevents lipid efflux. They proposed that sequestration of BSP proteins in seminal plasma by EY-LDF represent the major mechanism of sperm protection by EY. This experiment was planned to investigate the effect of interaction of EY with HBP on the freezability of buffalo cauda epididymal spermatozoa. For this study, comparisons were made between groups of spermatozoa, which had been treated with HBP in the absence of egg yolk and those, which had been treated with HBP pre-incubated with EY. We hypothesized that if EY-LDF was providing protection to sperm by sequestering HBP as proposed, then there would be much lower cryodamage in the group treated with HBP-EY mixture than in the group treated with HBP in absence of EY.

2. Materials and methods

2.1. Heparin binding protein (HBP)

HBP in buffalo seminal plasma were isolated using heparin-sepharose affinity chromatography as described earlier (Harshan et al., 2006). The purified proteins were lyophilized and stored at −20 °C.

2.2. Epididymal semen collection and extension

Twelve pairs of epididymis from adult buffalo bulls slaughtered at the National Buffalo Slaughter House, Bareilly (UP) were utilized for collection of epididymal semen. Each pair was utilized for a single set of experiment. The spermatozoa obtained by puncturing the epididymis at multiple sites were collected in Tris dilutor (Tris(hydroxy methyl) amino methane, 3.028 g; citric acid monohydrate, 1.675 g; fructose, 1.25 g; penicillin G sodium, 500–1000 IU/ml; streptomycin sulphate 500–1000 μg/ml; double glass distilled water up to 100 ml). The spermatozoa collected from the same pair of testes were mixed and the suspended semen sample was centrifuged at