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Effect of chondroitin sulfate C on sperm capacitation and fertilization parameters *in vitro* in pigs

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Abstract

The objective of this study was to determine the effects of chondroitin sulfate C (CS-C) on sperm capacitation and fertilization parameters *in vitro* in pigs. Frozen–thawed ejaculated pig sperm (semen S-484) were incubated with fertilization medium containing CS-C (0–2 mg/ml) for 1 h and the capacitation rate with chlorotetracycline (CTC) assay was examined, which showed that CS-C increased the rate of incapacitation F pattern spermatozoa converted to capacitation B pattern sperm cell in concentration-dependent manner and mostly increased capacitation B pattern sperm cell and decreased acrosome reaction AR pattern sperm cell in 1 mg/ml concentration. When sperm was incubated for 1, 2 and 4 h in fertilization medium containing 1 mg/ml CS-C, it showed that the capacitated B pattern sperm cell was significantly ($p < 0.01$) increased and the AR pattern sperm cell was significantly decreased at each time point in the presence than in the absence of CS-C. For identifying the validity of CS-C in sperm capacitation, sperm–oocyte was inseminated in fertilization medium containing CS-C (0–2 mg/ml) and the rate of fertilized oocytes was examined, which showed that the penetration rates significantly ($p < 0.05$) increased from 0.5 to 1.0 mg/ml concentrations (87.4–96.3%) compared with control (74.9%). For identifying the universality of CS-C in sperm capacitation, four different semens (boar S-484, S-454, D-815 and D-748) were incubated in fertilization medium containing CS-C (1 mg/ml) for 2 h, respectively, which showed that CS-C increased the rate of capacitation B pattern sperm cell and decreased acrosome reaction AR pattern sperm cell in each semen. And it showed that CS-C yielded a higher promote effect (93.9%, 83.9%, 60.7% and 44.9%, respectively) on sperm penetration compared to unaddition control (63.4%, 22.0%, 3.3% and 3.3%, respectively). Sperm–oocyte binding analysis showed that CS-C increased the number of sperm bound to oocyte compared unaddition

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control in each semen. These results suggested that CS-C is the efficient factor on sperm capacitation in pigs, CS-C may promote sperm from the incapacitated to capacitated state and sequentially prevent sperm from spontaneous acrosome reaction, and thus facilitate the sperm–zona binding and sperm penetration to oocyte.

Keywords: Chondroitin sulfate C; Sperm capacitation; *In vitro* fertilization; Pig oocytes

1. Introduction

In mammals, freshly ejaculated sperm are incapable of fertilizing the egg. They gain this ability during their transit through the female reproductive tract, and this process is called capacitation (Yanagimachi, 1994). The mechanism of capacitation is poorly understood, but some investigation suggested that it involves many biochemical changes, which include the removal of adsorbed components from the sperm surface, a change in membrane lipid composition, increase in permeability to certain ions such as Ca^{2+}, a change in internal pH, an increase in plasma membrane fluidity (Yanagimachi, 1994; Langlais and Roberts, 1985; Handrow et al., 1989; Parrish et al., 1993) and a decrease in the membrane cholesterol:phospholipid ratio (Langlais and Roberts, 1985; Parks and Ehrenwald, 1990). All these changes induce the redistribution of membrane components and thus increase sperm to hyperactivation and to undergo the acrosome reaction (AR) following interaction with the zona pellucida, the egg's extracellular matrix (Florman and Babcock, 1991; Kopf and Gerton, 1991).

Many investigations reported that glycosaminoglycans (GAGs) in female reproductive tract fluid may play a major role in sperm capacitation process, such as in estrous cows (Anderson and Killian, 1994; Grippo et al., 1995; Therien et al., 2005; Berqqbist et al., 2006), dogs (Kawakami et al., 2000) and humans (Eriksen et al., 1994, 1997; Hamamah et al., 1996). It has been proved that the GAGs present in female reproductive tract fluid are composed of chondroitin sulfate (CS), hyaluronic acid (HA) and heparan (HP). Addition of CS (Lenz and Martin, 1988; Miller and Hunter, 1986), HA (Shamsuddin et al., 1993), or HP (Handrow et al., 1982; Mahmoud and Parrish, 1996; Parrish et al., 1988a,b) to bovine sperm medium has been found to stimulate motility and capacitation of sperm. Among these GAGs, the HP was the strongest promoting factor in bovine sperm capacitation. It is a result of HP binds to sperm (Parrish et al., 1988a,b) and induces changes in the intracellular environment of the sperm. This results in Ca^{2+} uptake and an increase in intracellular free calcium and intracellular pH (Handrow et al., 1989; Parrish et al., 1993). Another change associated with HP-induced capacitation in bovine sperm is an increase in protein phosphorylation (Galantino-Homer et al., 1997). However, the fact that HP does not appear to be involved in the capacitation of pig spermatozoa or promote sperm penetration *in vitro*, and its reverse strongly inhibit sperm penetration *in vitro* (Wang et al., 1991; Kim et al., 1997). It is suggested that HP is not a universal capacitation agent in mammal and that other GAGs may be involved in sperm capacitation in pig.

The porcine follicular fluid contains various GAGs, the major constituent of which was identified as chondroitin sulfate, for it was found that chondroitinase A, B and C hydrolyzed 90% of the GAGs produced by the granulosa cells (Yanagishita and Hscall, 1979; Yanagishita et al., 1979). Cumulus–oocyte complexes and follicular fluid are released into the oviduct just at the time of ovulation (Hansen et al., 1993). The GAGs are also found in oviductal and uterine fluid (Ax and Ryan, 1979; Lee and Ax, 1984). It is highly probable that chondroitin sulfate derived