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Direct contact between boar spermatozoa and porcine oviductal epithelial cell (OEC) cultures is needed for optimal sperm survival in vitro

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ABSTRACT

Oviductal epithelial cell (OEC) co-culture prolongs sperm viability and motility in vitro in a number of species including humans and horses. This study has sought to determine the effects of homologous OEC co-culture on boar sperm function. To determine whether the effects on spermatozoa were specifically caused by co-culture with or by OEC secretions, or by both factors together, a number of co-culture and cell-conditioned medium (CM) experiments were conducted. Firstly, Percoll-washed spermatozoa were co-cultured with OECs and pig kidney epithelial (LLC-PK1) cells, and in medium without cells. Secondly, Percoll-washed spermatozoa were incubated with CM derived from both OECs and LLC-PK1 cells and in unconditioned medium. A number of sperm function parameters were assessed after 5, 30, 60, 90, 120, and 180 min, and 24 h of co-culturing or incubation with CM. Of all the sperm function parameters investigated, the percentage (%) viability data yielded the most interesting results. OECs (mean ± S.E.M.; 31.2 ± 1.10) were better than LLC-PK1 cells (24.3 ± 0.93) at prolonging the viability of unbound spermatozoa after 24 h of co-culturing ($P < 0.05$). Also after 24 h, the viability of spermatozoa bound to the OECs (77.6 ± 1.83) was significantly higher than in the case of the LLC-PK1 cells (53.5 ± 1.43; $P < 0.001$). Other sperm function parameters, e.g., capacitation and motility, were also influenced by OEC co-culturing and incubation with CM, although to a lesser degree. In

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conclusion, porcine homologous OEC co-culture and CM incubation specifically affect sperm function. However, we propose that it is OEC co-culturing, rather than OEC-CM, that has the greater influence.

1. Introduction

The oviduct plays a significant role in mammalian reproductive processes by providing an appropriate microenvironment for gamete transport, fertilization and early embryonic development (for review, see Hunter, 2005). In most mammals, including pigs, it is thought that sperm-oviductal epithelial cell (OEC) contact is one of the final phases of maturation and gives spermatozoa the ability to penetrate oocytes (Hunter, 1984).

Studies of sperm-OEC co-culture have been performed on several species: humans (Zhu et al., 2001), sheep (Gutiérrez et al., 1993), dogs (Kawakami et al., 2001), rats (Cortés et al., 2004), cattle (Gualteri and Talevi, 2003; Kodithuwakku et al., 2007) and horses (Dobrinski et al., 1996). Interestingly, the reports in the literature investigating the effects of homologous OEC co-culturing on boar spermatozoa have focused on spermatozoa-OEC binding (Suárez et al., 1991; Fazeli et al., 1999; Petrunkina et al., 2001a) and on the modulation of sperm function by apical membrane preparations (Fazeli et al., 2003).

Several authors have shown that human OECs prolong sperm survival, enhance sperm viability and motility, stabilize the acrosome, induce capacitation, and modify the frequency of tail beat (Kervancioglu et al., 1994; Morales et al., 1996). It has also been shown that co-culturing fresh or cryopreserved human sperm with bovine OECs stabilizes the sperm chromatin structure (Ellington et al., 1998).

In vivo, OECs secrete proteins into the oviductal fluid. Oviductal fluid proteins that bind to the sperm membrane certainly have a beneficial effect on human (Yao et al., 2000), bovine (Bergqvist et al., 2006) and stallion (Ellington et al., 1993) sperm function by capacitating the spermatozoa and inducing their hyperactivation. In humans, Quintero et al. (2005) proposed that oviductal fluid proteins stabilize the acrosome, thereby preventing premature acrosome reactions (AR) in the absence of the female gamete.

In similar fashion to the in vivo situation, OECs cultured in vitro secrete proteins into their surrounding growth medium. This medium is therefore said to be conditioned by the OECs and is often referred to as OEC-conditioned medium (CM). OEC-CM, like OEC co-culture, prolongs sperm survival, as has been shown in humans (Zhu et al., 2001) and bovines (Ijaz et al., 1994; Abe et al., 1995).

Despite the benefits of OEC-CM in terms of sperm function evidenced for other species, the reports in the literature that investigate the effects of homologous OEC-CM on boar sperm function are often only concerned with its effect on in vitro fertilization (IVF), with analyzing the number of oocytes reaching the two-pronucleus stage, and the polyspermy rate (Bureau et al., 2000). This being the case, the present study sought to determine the effects of homologous OEC co-culturing and CM on several different sperm function parameters including viability, motility, acrosome, mitochondrial sheath integrity and capacitation status over a 24 h period. In the co-culture experiments, two populations of spermatozoa were analyzed: those bound to, and those that remained unbound from, the OECs. Pig kidney epithelial (LLC-PK1) cells were used as a positive control for both the co-culture and CM experiments. Additionally, media without cells and unconditioned media were used as negative controls for the co-culture and CM experiments, respectively.

2. Materials and methods

All reagents and materials were obtained from Sigma–Aldrich® (St. Louis, MO, USA) unless stated otherwise.